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ORIGINAL ARTICLE

Identification of genomic regions associated with partial resistance to Aphanomyces root rot in pea

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Abstract

Root rot caused by *Aphanomyces euteiches* is a major concern in pea (*Pisum sativum* L.). The lack of other effective control strategies makes crucial the development of resistant varieties. Although partial resistance has been reported, its quantitative inheritance, the association of resistance-linked genomic regions with unfavorable agronomic traits, and the limited understanding of soil pathogen populations hinder its progress in breeding programs. To search for alternative genomic regions associated with this partial resistance, a genome-wide association study (GWAS) was performed on a pea collection not yet explored for *A. euteiches* resistance in genetic studies. The 323 accessions of the collection were inoculated with RB84 isolate, and foliar and root symptoms were assessed 20 days after inoculation. The performed GWAS revealed 27 significantly associated markers among 26,045 SilicoDArT and 7033 single-nucleotide polymorphism marker datasets. Detected markers were distributed along the seven pea chromosomes, with 12 within previously described quantitative trait loci (QTLs). Chromosomes 2 and 5 harbored a significant number of associated markers, identified here for the first time, highlighting promising regions for future investigation. Twenty-one candidate resistance genes were identified. This study uncovers new genomic regions linked with *A. euteiches* resistance and provides molecular markers and candidate genes to support precision breeding. Newly identified QTL may be more effective against specific isolates than known QTL, enabling improved QTL rotation in the field.

Abbreviations: AMP, adenosine monophosphate; BLINK, Bayesian information and linkage disequilibrium iteratively nested keyway; BLUEs, best linear unbiased estimates; BLUPs, best linear unbiased predictors; DArTSeq, diversity array technology sequencing; FarmCPU, fixed and random model circulating probability unification; FDR, false discovery rate; FSI, foliar symptoms index; GWAS, genome-wide associations study; LD, linkage disequilibrium; MAF, minor allele frequency; MBV, metalaxyl-benomyl-vancomycin; MLM, mixed linear model; MMLM, multiple locus mixed linear model; PR, partially resistant; QTL, quantitative trait locus; RRI, root rot index; SAM, significantly associated marker; SNP, single-nucleotide polymorphism.

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Plain Language Summary

Like all crops, peas can be affected by pathogens that reduce yield. One of them is *Aphanomyces euteiches*, which produces root rot. Chemical treatments are not effective against this pathogen, making the development of resistant pea plants a much more eco-friendly and effective control strategy. To achieve this goal, the first step is identifying the genes conferring resistance. With this objective, a diverse collection of 323 pea accessions, not explored before in this type of genetic study, was analyzed using genome-wide association studies, which connect plant symptomatology with its genome variation, allowing the identification of genomic regions involved in resistance. This research allowed the identification of putative genes associated with Aphanomyces resistance that can be used in the development of root rot-resistant peas.

1 | INTRODUCTION

Pea (*Pisum sativum* L.) is one of the most cultivated legumes globally. It provides a valuable source of protein and essential nutrients for both human and animal consumption. Moreover, its capacity to fix atmospheric nitrogen through symbiosis with *Rhizobium leguminosarum* makes it a key crop for sustainable agriculture systems (Ditzler et al., 2021; Iannetta et al., 2021). However, like any other crop, it is subject to various biotic and abiotic stresses that hinder its production (Bagheri et al., 2023; Rubiales et al., 2023).

Root rot caused by *Aphanomyces euteiches* is a major concern in pea production (Wu et al., 2018). This oomycete has been reported in four of the five continents, excluding Africa, causing devastating damage in pea fields (Becking et al., 2022). The ability of this pathogen to adopt resting structures, called oospores, that could survive over a decade in soils without a host, as well as its capacity to infect other legume species, makes its management difficult (Wu et al., 2018). *Aphanomyces euteiches* causes necrosis of the root system and softening of the epicotyl as primary symptoms, resulting in wilting and chlorosis of plant shoots (Hughes & Grau, 2007). This pathogen presents high genetic diversity. Consequently, *A. euteiches* isolates have been classified into 11 virulence types, called pathotypes, based on their reaction to six pea accessions: Baccara, Capella, MN313, 90-2131, 552, and PI 80693 (Wicker et al., 2003). Pathotype I isolates are virulent in the whole set, whereas the remaining pathotypes show avirulence on specific accessions: PI 80693 (pathotype II), MN 313 (pathotype III), MN 313 and 552 (pathotype IV), 90-2131 and PI 80693 (pathotype V), all except Baccara (pathotype VI), 552 and PI 80693 (pathotype VII), MN 313, 552, and PI 80693 (pathotype VIII), Capella, 552, and PI 80693 (pathotype IX), 90-2131, 552, and PI 80693 (pathotype X), and 90-2131 and 552 (pathotype XI) (Sivachandra Kumar et al., 2021). Among these, pathotypes I and III are the most prevalent in infested soils, with pathotype I predominant in Europe and pathotype III predominant in Canada and the United States (Moussart et al., 2024).

Several management strategies have been proposed to deal with this pathogen, but none of them provides a complete solution (Wu et al., 2018). The most common approaches include crop rotation with non-host species and the use of predictive tests to detect and quantify *A. euteiches* prior to sowing, thereby avoiding sowing when necessary (Vandemark et al., 2000). The development of resistant varieties appears as a promising sustainable, and efficient solution to this problem. Although some accessions with partial resistance have been described, progress in resistance breeding has been hindered by (i) the polygenic inheritance of *A. euteiches* resistance, (ii) the link of the genomic regions associated with the resistance with agronomically undesired characteristics, and (iii) the limited knowledge of the pathogen biology as well as the diverse composition of infested soil populations (Hamon et al., 2013; Lavaud et al., 2024; Pilet-Nayel et al., 2002). However, the pathogen's ability to adopt resistance structures, its mixed reproductive system combining sexual and asexual stages, and the genetic diversity of its populations highlight partial resistance, which imposes a more diversified and reduced pressure on the pathogen populations, as the most reliable and durable strategy to manage it (McDonald & Linde, 2002; Mundt, 2014). Searching for alternative, more attractive breeding genetic regions associated with this trait could help overcome current breeding limitations and enable the rotation of quantitative resistance genes at the field scale.

Genetic linkage analysis using recombinant inbred lines and advanced backcross, along with genome-wide association studies (GWAS) conducted across diverse populations, environments, and different *A. euteiches* strains and pathotypes, allowed the identification of quantitative trait loci (QTLs) associated with resistance across the seven pea chromosomes, highlighting the quantitative and complex nature of the resistance against this pathogen in pea (Wu et al., 2021). Ten of these QTLs, *Ae-Ps1.2*, *Ae-Ps2.2*, *Ae-Ps3.1*, *Ae-Ps3.2*, *Ae-Ps4.1*, *Ae-Ps4.3*, *Ae-Ps4.5*, *Ae-Ps5.1,* *Ae-Ps5.2*, and *Ae-Ps7.6*, were consistently associated with pea resistance in diverse environments and populations, although their effect varied depending on the pathotype of the tested isolate (Desgroux et al., 2016; Hamon et al., 2011, 2013; Lavaud et al., 2024; Leprévost et al., 2023; Pilet-Nayel et al., 2002; Wu et al., 2021). They were associated with genes implicated in plant defence, such as leucine zipper proteins (*Ae-Ps1.2*), protein kinases (*Ae-Ps3.1* and *Ae-Ps4.1*), DED/DEAH box helicase (*Ae-Ps4.5*), and putative stress-related genes (*Ae-Ps7.6*), proposed as implicated in pea plant response against *A. euteiches* (Desgroux et al., 2016; Hamon et al., 2013; Lavaud et al., 2024).

The present study aims to increase the knowledge of pea resistance to Aphanomyces root rot by identifying new genetic regions associated with partial resistance using a GWAS approach with pea germplasm not explored before in this type of genetic study. For this purpose, the response of a globally diverse pea panel to *A. euteiches* was assessed. Phenotypic data were associated with molecular markers generated via Diversity Arrays Technology sequencing (DArTseq), enabling the identification of genomic regions significantly associated with resistance. Subsequent mapping of associated markers onto pea reference genomes revealed candidate genes potentially involved in the resistance response.

Core Ideas

- Natural variation in pea response to Aphanomyces root rot provides insight into the genetic basis of resistance.
- Molecular markers were associated with pea response to Aphanomyces root rot.
- New genomic regions associated with Aphanomyces root rot partial resistance in pea.
- Identification of putative genes involved in the response co-located with significantly associated markers.

2 | MATERIALS AND METHODS

2.1 | Phenotypic screening

2.1.1 | Plant material and experimental design

A 323 *Pisum* spp. accessions collection described by Rispail et al. (2023) was used in this study. This collection was selected from a large *Pisum* spp., one of >3000 accessions initially gathered from USDA (Department of Agriculture, USA), JIC (John Innes Center, UK), CRF-INIA-CSIC (Centro Nacional de Recursos Fitogenéticos, Spain), CGN (CPRO-DLO, the Netherlands), IPK (Leibniz Institute of Plant Genetics and Crop Plant Research, Germany), and ICARDA (International Center for Agricultural Research in the Dry Areas, Syria). The studied worldwide collection included various *Pisum* species (296 *P. sativum*, 16 *P. fulvum*, 13 *P. abyssinicum*, and 1 *P. jomardi* accessions) and material types (168 landraces, 53 cultivars, 44 wilds, 10 breeding lines, and 45 unknown).

For phenotype screening, seeds were sterilized with a 0.1% bleach solution, scarified, and pregerminated in petri dishes with wet paper for 3 days in darkness at 23 ± 2˚C. Germinated seeds were sowed in 200-mL plastic pots (6 × 6 × 10 cm) with autoclaved perlite (one seed per pot). Pots were placed in plastic trays (8 × 6 pots per tray) in controlled conditions (growth chamber at the Institute of Sustainable Agriculture, 24 ± 2˚C, 65 RH, 12:12 h D:N with 150 µmol m^{-2} s^{-1}). Plants were watered every 3–4 days with Hougland solution (Hoagland & Arnon, 1938).

The inoculation trial was conducted using a randomized complete block design and repeated three times at different rounds. Each round included four plants (replicates) of each accession, randomly distributed in four different trays (blocks). One plant of pea Messire was included in each tray as susceptible control.

2.1.2 | *Aphanomyces euteiches* growth and inoculation

Plants were inoculated with the *A. euteiches* pathotype I isolate, RB84 (France), kindly provided by Marie–Laure Pilet–Nayel from INRAE (French National Institute for Agriculture, Food, and Environment, Rennes, France) (Moussart et al., 2006). The isolate was grown in metalaxyl-benomyl-vancomycin (MBV) medium for 7 days at 24˚C in darkness before zoospore production.

Zoospore production was carried out following the method described by Rodriguez-Mena et al. (2024). Approximately 20 plugs (5 × 5 mm) of the MBV *A. euteiches* culture were transferred to a 200 mL Erlenmeyer flask that contains 50 mL of peptone-glucose broth (20 g/L of peptone and 5 g/L of glucose). Cultures were grown at 24˚C in darkness for 4 days. Subsequently, under sterile conditions, the peptone-glucose broth was removed, and the mycelium was washed twice with autoclaved water, allowing a 2-h interval between washes. Following the washing steps, 50 mL of mineral salt solution (0.26 g of $CaCl_2{\cdot}2H_2O{\cdot}0.07$ g of KCl, and 0.48 g of $MgSO_4{\cdot}7$ H_2O dissolved in 1 L of distilled autoclaved water) was added to the mycelium, and the culture was incubated in darkness at

24°C for 16 h to induce zoospore production. The mycelium was then filtered, and, using a Fuchs–Rosenthal chamber, the zoospore concentration in the salt solution was calculated and adjusted to 1000 zoospores/mL. Finally, 10-day-old plants were inoculated by pipetting 5 mL of the zoospore suspension at the base of the stem.

2.1.3 | Symptoms evaluation

Twenty days after *A. euteiches* inoculation, foliar (foliar symptoms index, FSI) and root symptoms (root rot index, RRI) were assessed. Foliar symptoms were evaluated on a 0–5 scale (Rodriguez-Mena et al., 2024), where 0 = healthy plants with no symptoms; 1 = slight wilting of plant leaves with 10%–25% of the aerial part affected; 2 = moderate wilting, 26%–50% of the aerial part affected; 3 = disease progression affecting more than half of the aerial part, 51%–75% of the aerial part affected; 4 = extensive discoloration of the part, 76%–90% of the aerial part affected; 5 = completely dead plant. Root symptoms were assessed on a 0–9 scale; 0 = completely healthy roots; 1 = light yellowing, infested area <5%; 2 = slight necrosis or few small lesions on lower stem, infested area 5%–10%; 3 = moderate necrosis or small lesions scattered over the lower stem, infested area 11%–25%; 4 = moderate necrosis or small lesions scattered over the lower stem, infested area 26%–50%; 5 = extensive necrosis with large lower stem damage, infested area 51%–75%; 6 = extensive necrosis with large lower stem damage, infested area 75%–100%; 7 = complete necrosis with large lower stem damage and significantly reduced root development; 8 = complete necrosis with large lower stem damage and scarce root development; 9 = completely dead plant (Wu et al., 2018; Xue, 2000).

2.1.4 | Phenotypic data statistical analysis

Phenotypic data statistical analyses were conducted using R software version 4.4.2 (R Core Team, 2024) with ggplot2 package for data visualization (Wickham, 2016). FSI and RRI phenotypic data were analyzed independently. First, outliers were identified using the interquartile range method and removed when required (Estaghvirou et al., 2014; Tripodi, 2022). Then the effect of the experimental components (round, replicates, and genotypes) on the scoring indices was assessed with a Kruskal–Wallis test due to the ordinal nature of the data (*kruskal.test* function from the "stats" package [R Core Team, 2024]).

Values of both scoring indices were analyzed using a cumulative link mixed model with logit as link function (*clmm* function from the "ordinal" package (Christensen, 2019; Leprévost et al., 2025). Two models were tested: a mixed model and a random model. The mixed model adjustment was: *Scoring index* (FSI/RRI) = *G* (fixed term) + Rd + Rd:G + Rd:Repl (random terms), where G represents each of the 323 evaluated pea accessions, Rd each of the three rounds, Repl each of the four plants per accession included in each repetition, and Rd:G and Rd:Repl the round by genotype and the round by replicate interaction, respectively. The random model followed the same structure, but with the genotype term also as random. Finally, best linear unbiased estimates (BLUEs) were obtained from the mixed model (*emmeas* function of the emmeans package (Lenth et al., 2025)) and best linear unbiased predictors (BLUPs) from the random model (*ranef* function from "lm4" package [Bates et al., 2025]) (Table S1).

In addition, broad-sense heritability (H^2) of both scoring indices was calculated from the variances of the random model components using the formula:

$$H^2 = \frac{\sigma_\mathrm{G}}{\sigma_\mathrm{G} + \sigma_\mathrm{E}/r}$$

where σ_G and σ_E, represent the genetic and the residual variances, respectively, and r the number of replicates (Estaghvirou et al., 2014; Leprévost et al., 2025). Pearson correlation coefficient between means of the original FSI and RRI values was also calculated (*stat_poly_eq* function from ggpmisc package [Aphalo et al., 2024]).

2.2 | Genotypic screening

2.2.1 | Data acquisition

For the DNA extraction, 20 third leaves of 2-weeks-old plants grown in controlled conditions were pooled together per accession, flash frozen in liquid nitrogen, and freeze-dried. DNA was isolated from the frozen pool of leaves using a modified cetyltrimethylammonium bromide (CTAB)/chloroform/isoamyl alcohol method (Doyle & Doyle, 1987). Quality was assessed by electrophoresis on a 0.8% agarose gel. Subsequently, DNA was quantified by fluorescence art 504 $\mathrm{nm_{Ex}}$/531 $\mathrm{nm_{Em}}$ on an HT Synergy microplate reader (Biotek, Winnoski) with Quantifluor DsDNA system (Promega Corporation) following the manufacturer's instructions. Finally, DNA concentration was adjusted to 20 ng/µL.

Genotyping was performed with a SilicoDArT approach by Diversity Arrays P/L using the high-diversity Pea DarT-Seq 1.0 array (50,000 markers), adapted for wild *Pisum* spp. accessions. Complexity reduction with the *Pst*I and *Mse*I restriction enzymes, library constructions, amplification, and Illumina sequencing were performed as described by Barilli et al. (2018). This process yielded two markers datasets; presence-absence sequences variants (SilicoDArT) and single-nucleotide polymorphism (SNP) (Rispail et al., 2023).

2.2.2 | Quality control and mapping

To ensure high data quality, markers with >20% missing data, minor allele frequency (MAF) below 5%, or heterozygosity rate above 10% were removed. The retained markers were subsequently mapped using BLASTn (E-value $< 1 \times 10^{-5}$; word size = 11) (Camacho et al., 2009) into the *Pisum sativum* reference genomes (Cameor v1a and ZW6 v1.0), prioritizing their positions in Cameor to be consistent with previous works in this field (Kreplak et al., 2019). When no corresponding position was available in Cameor, ZW6 was used as an alternative (Yang et al., 2022). Markers with identical alignment quality mapped in different chromosomes or located in different chromosomes in the two reference genomes, scaffolds located or unmapped and aligned, were classified as “unmapped” with a randomly assigned position.

2.2.3 | Linkage disequilibrium (LD) calculation

Following a similar process as described by Sampaio et al., 2021, a SilicoDArT subset of 4575 markers was chosen, considering a 0.5 Mbp interval between markers within each chromosome. For SNP markers the interval distance was reduced to 0.1 Mbp, resulting in a 4067 SNP marker subset. These subsets were used to calculate principal components to determine the population structure. LD was estimated for all the mapped markers as the squared coefficient of correlation between marker pairs (r^2), after correction for population structure using the principal component scores from Eigenanalysis. These analyses were performed in Genstat software, 24th ed. (VSN).

2.3 | Genetic base of partial resistance

2.3.1 | Genome-wide association study

The cleaned and mapped SilicoDArT and SNP markers were used to perform GWAS with both scoring indices across the 323 evaluated pea accessions using GAPIT 3.0 (Wang & Zhang, 2021). Due to the different nature of the markers, independent GWASs were performed. With each type of genomic marker (SilicoDArT and SNP), a single-trait GWAS was conducted with both scoring indices testing BLUEs and BLUPs adjustment and four different models: single-locus mixed linear model (MLM), multiple-locus mixed linear model (MMLM), multi-locus Bayesian information and LD iteratively nested keyway (BLINK), and fixed and random model circulating probability unification (FarmCPU) to determine which adjustment and method suited better to the data (Wang et al., 2022). To optimize GAPIT performance as well as control the population structure of the collection, Kinship matrix was calculated using GAPIT, indicating “VanRaden” as method (argument *Kinship.algorithm*), and PCA was included with *model.selection* argument to ensure the optimal number of principal components to include in each phenotype trait based on the Bayesian information criterion (Zhang, 2023).

For each tested adjustment and model, quantile-quantile plots were depicted to assess the overall distribution of the p-values. To evaluate the genomic inflation or deflation of each model, lambda (λ) was calculated using the *estlambda2* function of the QQperm package (Wang, 2016). Based on λ values, the best-performing model and phenotypic adjustment (BLUEs or BLUPs) were selected for each scoring index and marker type.

Significant associated markers (SAMs) were identified from the selected model using false discovery rate (FDR) corrections, with a significance threshold of adjusted p-value of 0.05 (Chen et al., 2021). Compared to the conventional Bonferroni correction, FDR provides a less conservative approach, allowing the identification of potentially interesting markers (Osuna-Caballero et al., 2024; Storey & Tibshirani, 2003). p-value adjustments were performed using the *p.adjust* function of the stats package (R Core Team, 2024).

Phenotype variance estimation was also performed for the SAMs using the formula: $V_{\mathrm{QTL}}/V_{\mathrm{Phenotype}}$, in which $V_{\mathrm{Phenotype}}$ represents the phenotype variance (*var* function of stat package [R Core Team, 2024]) and V_{QTL} the variance of the marker calculated as $2 \times \mathrm{MAF} \times (1\text{-}\mathrm{MAF}) \times \mathrm{effect}^2$ (Resende et al., 2016).

2.3.2 | Partially resistant major allele frequency

Based on FSI and RRI, the 323 pea accessions evaluated were classified into three phenotypic response groups. For FSI, groups were defined as partially resistant (PR) (FSI ≤ 2), moderately susceptible ($2 <$ FSI ≤ 4), and susceptible ($4 <$ FSI ≤ 5). For RRI, groups were: PR (RRI ≤ 6), moderately susceptible ($6 <$ RRI ≤ 7) and susceptible ($7 <$ RRI ≤ 9).

In each SAM, the most frequent allele within the PR associated group was designated as “partially resistant major allele” (PR major allele). Subsequently, the frequency of the PR major allele was calculated for each SAM. If the SAM was a SilicoDArT marker, PR major allele frequency was calculated as (number of accessions with the PR major allele)/(total number of accessions in the group). If the SAM was an SNP marker, the PR major allele frequency was calculated as (2 × number of homozygotes + number of heterozygotes)/(2 × total number of accessions in the group).

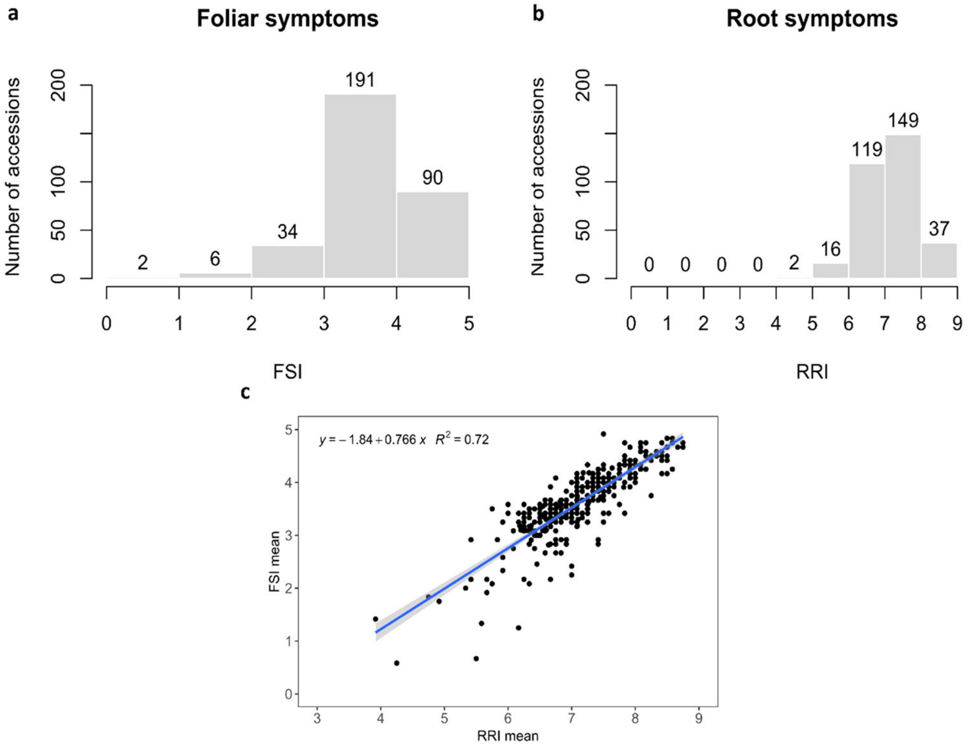

FIGURE 1 Frequency distribution of average foliar symptoms index (FSI: scale 0–5) (a), average root rot index (RRI: scale 0–9) (b) and correlation between both scoring index (c) for the 323 evaluated pea accessions 20 days after the inoculation with *Aphanomyces euteiches* under controlled conditions.

2.3.3 | Candidate genes

For each SAM, a local LD inspection was performed to define chromosomal regions to search for candidate genes. Based on the previously calculated LD, nearby markers were checked and considered linked when $r^2 > 0.2$. The search window for candidate genes was initially restricted to the SAM position. However, if the SAM showed linkage to additional markers, the window was extended to encompass the region between the SAM and its linked markers (Leitão et al., 2023).

Candidate gene annotation was performed manually using the JBrowser tool from https://urgi.versailles.inra.fr/ for the two pea reference genomes (Cameor v1a and ZW6 v1.0). Functional characterization was obtained using MapMan4 web tool Mercator4 V2.0 (https://mapman.gabipd.org/app/mercator) (Schwacke et al., 2019).

3 | RESULTS

3.1 | Phenotypic response to the infection, variance components, and heritability

A wide range in foliar and radical response to *A. euteiches* infection was observed among the 323 evaluated pea accessions (Figure 1a,b). Based on FSI, eight accessions were grouped as PR (FSI $\leq$ 2), 225 as moderately susceptible (2 < FSI $\leq$ 4), and 90 as susceptible (FSI > 4). Regarding RRI, 18 accessions were grouped as PR (RRI $\leq$ 6), as moderately susceptible 119 (6 < RRI $\leq$ 7), and 186 as susceptible (RRI $\leq$ 9). A considerable correlation between FSI and RRI was observed ($R^2 = 0.72$) (Figure 1c).

Both scoring indices were influenced by genotype and round factors (Kruskal–Wallis test p-value < 0.05). However, the genotype effect was markedly stronger than either round or round-genotype interaction effects, which is also supported by a high broad-sense heritability for both indices ($H^2 > 0.90$) (Table 1). These facts, along with the homogeneity between rounds, indicate that the genotypic component is the main determinant of the observed phenotypic differences, confirming the suitability of using the average values from the three rounds in the GWAS (Figure S1; Table S2).

3.2 | Genotypic data

The genotyping process yielded 66,643 SilicoDArT and 55,269 SNP markers. After quality filtering, 26,045 SilicoDArT and 7033 SNP high-quality markers were retained. Of these, 23,639 SilicoDArT and 6744 SNP markers were successfully mapped in pea reference genomes (Cameor position prioritized), providing comprehensive coverage across

TABLE 1 Variance components and broad-sense heritability (H^2) for foliar (FSI) and root (RRI) indices, 20 days after *Aphanomyces euteiches* inoculation, in the 323 evaluated pea accessions.

Scoring index	Variance components				
	σ_G[a]	σ_{Rd}[b]	$\sigma_{Rd:G}$[c]	$\sigma_{Rd:Repl}$[d]	H^2[e]
FSI	1.69	0.02	0.39	0.04	0.94
RRI	1.44	0.08	0.42	0.03	0.92

[a] $\sigma_{G:}$ variance component of the genotype effect.
[b] σ_{Rd}: variance component of the three rounds.
[c] $\sigma_{Rd:G}$: variance component of the interaction between round and genotype effect.
[d] $\sigma_{Rd:Repl}$: variance component of the interaction between round and the four repetitions (plants of each accession) included in each of the rounds.
[e] H^2: broad-sense heritability.

the seven chromosomes (Figure S2). The number of markers per chromosome ranged from 2922 to 4377 for SilicoDArT markers and from 714 to 1347 for the SNP ones (Table S3).

3.3 | Best adjustment and model selection

BLUPs adjustment yielded better λ values compared to BLUEs, indicating a better adjustment for modeling (Table S4). Among the tested GWAS models, MLM, MMLM, and BLINK exhibited the highest inflation values, while FarmCPU showed the closest to 1 λ values and the lowest deviation in the calculated *p*-values from the expected ones on *Q–Q* plot graphs, indicating that this model provided the highest predictive power among those tested (Figures S3–S6). Based on this result, BLUPs and FarmCPU were selected as phenotypic adjustment and model to perform the GWAS with both scoring indices and marker types.

3.4 | Significant associated markers

The GWAS conducted using the FarmCPU model allowed the identification of 27 markers significantly associated with at least one scoring index. Of these, 17 were SilicoDArT and 10 SNP markers. In total, 14 markers were associated with foliar response (9 SilicoDArT + 5 SNP), 11 with radical response (8 SilicoDArT + 3 SNP), and two SNP SAMs were common between both scoring indices (Figure 2).

At least one SAM was identified on each of the seven pea chromosomes. Markers associated with foliar response were distributed across all chromosomes except chromosome 7, with chromosome 5 harboring the highest number of foliar SAMs. In contrast, markers associated with radical response were located on chromosomes 2, 5, 6, and 7, with chromosome 2 being the one that contained the largest number of SAMs for this scoring index. The two common markers between both scoring indices were located on chromosomes 2 and 5 (Table 2).

Each SAM accounted for only a small proportion of the observed phenotypic variance. The percentage of phenotypic variance explained individually by each FSI SAMs ranged from 1.52% to 4.25%. From RRI SAMs, the percentage of phenotypic variance explained individually ranged from 1.50% to 5.15% (Table 2).

3.5 | PR major allele frequency among groups

Not all the identified SAMs exhibited clear differences in PR major allele frequency among the phenotypic response groups. These differences were more evident for markers associated with the root scoring index than for those associated with the foliar scoring index. Among the 16 markers associated with foliar response, nine showed a higher frequency of the PR major allele in the PR group compared to the susceptible group (DART3538180, DART4660168, DART5898112, DART5945201, SNP3554107_2, SNP3563412, SNP3565318, SNP4663348, and SNP5957817). In contrast, in eight of the 13 SAMs associated with root response, the PR major allele presence in the PR group was significantly higher than in the susceptible group (DART3558494, DART3561046, DART5934328, DART5944388, DART5955376, SNP3564259, SNP3565318, and SNP5957817) (Table S5). Although the PR major allele of DART5898112 (associated with foliar response) was always present in the resistant category, its frequency was also high in the other categories. Among SAMs, the most pronounced differences between PR and other categories were observed for DART3538180, SNP4663348, and SNP5957817 associated with foliar response and DART5955376, SNP3564259, and SNP5957817 associated with root response (Figure 3). The percentage of SAMs that presented differences in PR major allele frequency in the PR group with respect to the susceptible group was similar for SilicoDArT (75.0%) and SNP (66.7%) markers.

3.6 | Candidate genes

Only three SilicoDArT SAMs were in LD with other markers. For these SAMs, the candidate gene search window was expanded to include the genomic region between the linked

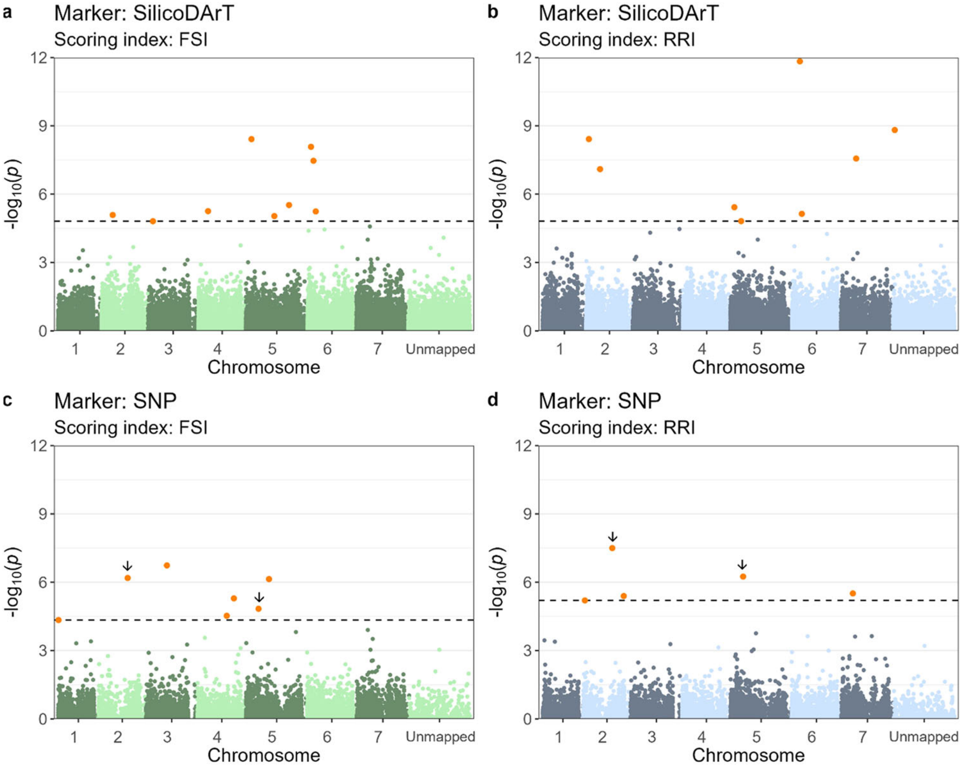

FIGURE 2 Manhattan plots of genome-wide association performed with 26,045 SilicoDArT and 7033 single-nucleotide polymorphism (SNP) markers and the foliar (FSI) and root (RRI) responses in 323 evaluated pea accessions, 20 days after the inoculation with *Aphanomyces euteiches*. The *x*-axis indicates the position of each marker across the seven pea chromosomes, including unmapped markers grouped under “unmapped.” The *y*-axis represents the $-\log_{10}$ of the *p*-value obtained from the genome-wide associations study (GWAS) using fixed and random model circulating probability unification (FarmCPU) model and best linear unbiased predictors (BLUPs) adjustment. Significant markers, selected based on the false discovery rate method (p-value < 0.05), are orange colored. The dashed horizontal line indicates false discovery rate significance threshold. Arrows indicate significant markers common between both scoring indices. Graphs a and b show SilicoDArT results for FSI and RRI, respectively, while c and d show SNP results for FSI and RRI, respectively.

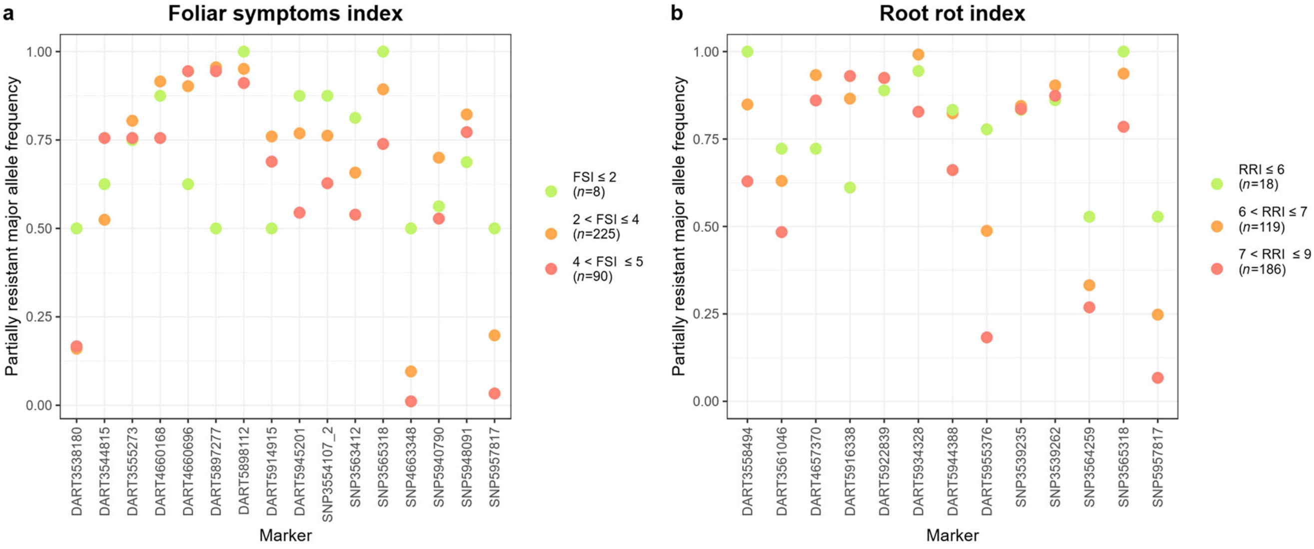

FIGURE 3 Frequency of the partially resistant major allele (more frequent in partially resistant group) for markers significantly associated with foliar scoring index (a) and root scoring index (b). The *x*-axis represents marker identifiers labeled with the prefix “DART” for SilicoDArT markers and “single-nucleotide polymorphism (SNP)” for SNP markers. The *y*-axis shows the frequency of the partially resistant major allele.

TABLE 2 Markers with significant association (false discovery rate [FDR] p-value < 0.05) with foliar (FSI) or root (RRI) evaluation in the 323 evaluated pea accessions, 20 days after the inoculation with *Aphanomyces euteiches*, indicating marker code, chromosome, position, p-values adjusted by the false discovery rate method, effect and minor allele frequency (MAF), and percentage of the phenotypic variance estimation (PVE).

Scoring index	Marker code[a]	Chromosome	Position (bp)	Adjusted p-value	Effect[b]	MAF	PVE (%)[c]
FSI	SNP948091	1	4,039,511	7.09×10^{-3}	−0.28	0.13	1.69
	DART4660168	2	117,305,897	1.09×10^{-4}	0.30	0.12	1.62
	SNP5957817*	2	300,392,669	7.09×10^{-3}	−0.36	0.24	4.25
	DART3544815	3	51,633,609	4.45×10^{-2}	−0.19	0.36	1.52
	SNP4663348	3	216,337,201	8.84×10^{-3}	−0.42	0.11	3.25
	DART3555273	4	101,616,918	2.48×10^{-2}	−0.24	0.20	1.64
	SNP3554107_2	4	302,688,911	1.28×10^{-3}	0.30	0.17	2.31
	SNP3563412	4	376,274,094	3.49×10^{-2}	0.23	0.34	2.19
	DART5897277	5	59,269,301	2.48×10^{-2}	−0.64	0.06	3.88
	SNP3565318*	5	129,746,720	2.06×10^{-2}	0.43	0.11	3.20
	SNP5940790	5	238,197,238	2.23×10^{-4}	0.26	0.32	2.63
	DART3538180	5	301,037,853	2.30×10^{-2}	−0.24	0.19	1.61
	DART5945201	5	456,689,274	1.77×10^{-4}	0.30	0.25	2.94
	DART4660696	6	36,410,600	2.97×10^{-4}	−0.52	0.08	3.65
	DART5914915	6	62,698,414	4.15×10^{-4}	0.30	0.25	3.08
	DART5898112	6	86,904,185	2.48×10^{-2}	0.57	0.05	2.96
RRI	SNP3564259	2	16,843,323	8.98×10^{-3}	−0.20	0.34	1.59
	DART3558494	2	27,923,574	9.99×10^{-5}	0.30	0.22	2.88
	DART5955376	2	146,655,305	1.99×10^{-5}	−0.24	0.36	2.35
	SNP5957817*	2	300,392,669	1.99×10^{-3}	−0.38	0.24	4.67
	SNP3539235	2	419,140,902	4.58×10^{-2}	−0.28	0.15	1.79
	DART5944388	5	40,807,975	2.71×10^{-2}	0.22	0.24	1.61
	DART5934328	5	112,571,109	3.84×10^{-8}	0.31	0.09	1.50
	SNP3565318*	5	129,746,720	1.70×10^{-3}	0.46	0.11	3.65
	DART5922839	6	81,273,613	4.97×10^{-2}	0.66	0.07	5.15
	DART5916338	6	101,925,279	1.63×10^{-2}	−0.34	0.10	1.90
	SNP3539262	7	120,443,323	1.70×10^{-3}	−0.35	0.11	2.28
	DART3561046	7	160,339,789	2.98×10^{-2}	0.21	0.41	1.90
	DART4657370	Unmapped	NA	1.96×10^{-2}	−0.42	0.11	3.08

[a] Single-nucleotide polymorphism (SNP) before the marker code indicates a SNP marker, and diversity array technology (DART) before the marker code indicates a SilicoDArT marker.

[b] The effect parameter indicates how much the presence of the minor allele in the marker increases (positive value) or decreases (negative value) the scoring index value.

[c] Phenotype variance estimation (PVE). Percentage of the amount of phenotypic variance explained by each association is calculated as $V_{QTL}/V_{Phenotype}$.

*Common significant associated markers between both scoring indices.

markers. In the other cases, the search was restricted to the marker location (Table S6).

To identify potential candidate genes, the chromosomal locations of the significantly associated markers (SAMs) were analyzed using Cameor and ZW6 genome annotations (Kreplak et al., 2019; Yang et al., 2022). Twenty-one of the 27 SAMs were associated with a gene. Among them, 11 were associated with foliar-related SAMs (7 SilicoDArT and four SNP), eight with root-related SAMs (6 SilicoDArT and 2 SNP), and two with the common SAMs between both scoring index (SNP) (Table 3).

Genes associated with foliar response were functionally diverse. A Snapin/Pallidin coding gene was associated with SNP5948091 on chromosome 1. Chromosome 3 SAM (SNP4663348) was associated with a gene that codified a phosphomannomutase. On chromosome 4, associated markers were located on genes encoding a centromere protein (DART3555273), a pre-mRNA splicing factor (SNP3554107_2), and a lipoxygenase (SNP3563412). Chromosome 5 harbored SAMs linked to genes encoding aminoacyl-tRNA synthetases (DART5897277) and cellulose synthase (DART3538180). On chromosome 6, two genes

TABLE 3 Candidate genes of pea response against *Aphanomyces euteiches* measured through the scoring indices (foliar symptomatology, foliar symptoms index [FSI], or root symptomatology, RRI), in the 323 evaluated pea accessions, 20 days after the inoculation with *Aphanomyces euteiches*.

Scoring index	Marker code	Chromosome	Gene ID[a]	Genome[b]	Description[c]
FSI	SNP5948091	1	*Psat1g002720*	Cameor	Snapin/Pallidin
	DART4660168	2	*Psat2g066000*	Cameor	Domain with unknown function
	DART3544815	3	NA	NA	NA
	SNP4663348	3	*Psat3g111000*	Cameor	Eukaryotic phosphomannomutase
	DART3555273	4	*Psat4g062040*	Cameor	Centromere protein S associated with centromere protein X
	SNP3554107_2	4	*Psat4g154840*	Cameor	Pre-mRNA-splicing factor of RES complex
	SNP3563412	4	*Psat4g184760*	Cameor	Lipoxygenase
	DART5897277	5	*Psat5g031520*	Cameor	TRNA synthetases class I (C) catalytic domain. Ligase forming carbon-oxygen bond
	SNP5940790	5	NA	NA	NA
	DART3538180	5	*Psat5g165160*	Cameor	Cellulose synthase
	DART5945201	5	NA	NA	NA
	DART4660696	6	*Psat6g041040*	Cameor	Chloroplast AMP-binding enzyme
	DART5914915	6	*Psat6g058040*	Cameor	Guanylate-binding protein; N-terminal domain
	DART5898112	6	*Psat06G0172900*	ZW6	Leucine-rich repeat hypothetical protein
RRI	SNP3564259	2	*Psat2g017120*	Cameor	Hypothetical protein. Anaphase promoting complex subunit
	DART3558494	2	NA	NA	NA
	DART5955376	2	*Psat02G0189600*	ZW6	Hypothetical protein. NADPH-dependent FMN reductase. Oxidoreductase acting on NADH or NADPH
	SNP3539235	2	*Psat2g180000*	Cameor	Clp amino terminal domain. Pathogenicity island component. Metabolite transporter
	DART5944388	5	*Psat5g021760*	Cameor	Exostosin family
	DART5934328	5	NA	NA	NA
	DART5922839	6	*Psat6g067240*	Cameor	Ribosomal protein L4/L1 family. Ribosome biogenesis large ribosomal subunit
	DART5916338	6	*Psat06G0190400*	ZW6	Hypothetical proteins: RNA recognition motif and RING/Ubox like zinc-binding domain
	SNP3539262	7	*Psat7g071560*	Cameor	AMP-binding enzyme
	DART3561046	7	*Psat7g097400*	Cameor	Glycosyl hydrolase family; 3 C-terminal domain
	DART4657370	Unmapped	NA	NA	NA
FSI and RRI	SNP5957817	2	*Psat2g114680*	Cameor	Hypothetical protein. Cofactor assembly of complex C subunit B, CCB2/CCB4. Photosynthesis and phosphorylation assembly factor (CCB4)
	SNP3565318	5	*Psat5g070840*	Cameor	No apical meristem protein. NAC transcription factor

Note: Including scoring index, significant marker code, chromosome in which the marker is located, associated gene ID, reference genome in which the gene was found, and description. Gene ID and description for markers located in non-coding regions are indicated as not available (NA).

Abbreviations: DART, diversity array technology; SNP, single-nucleotide polymorphism.

[a] URGI data base code in https://urgi.versailles.inra.fr/Species/Pisum.

[b] Pea's reference genome in which the gene was found.

[c] Gene description in URGI database (https://urgi.versailles.inra.fr/Species/Pisum) and Mercator functional annotation when available. The first sentence of the description corresponds to URGI database description and the second one to the functional annotation in Mercator.

were identified, encoding a chloroplast adenosine monophosphate (AMP)-binding enzyme (DART4660696) and a guanylate-binding protein (DART5914915), respectively. Additionally, two hypothetical proteins were identified, one on chromosome 2 (DART4660168) and another on chromosome 6 (DART5898112).

Regarding root-associated genes, they were identified across chromosomes 2, 5, 6, and 7. On chromosome 2, SAMs were associated with a gene that codified a protein related to mRNA degradation (SNP3564259), an oxidoreductase-linked gene (DART5955376), and a pathogenicity island component coding gene (SNP3539235). DART5944388 on chromosome 5 is located on a gene that codified a protein of the extensin family. On chromosome 6, SAM-associated genes encoded a protein implicated in ribosomal biogenesis (DART5922839) and a hypothetical protein (DART5916338). Finally, on chromosome 7, an AMP-binding enzyme (SNP3539262) and a glycosyl hydrolase protein (DART3561046) were the candidate genes linked to the SAMs located in this chromosome.

With respect to the common markers, both were associated with a candidate gene. SNP5957817, located on chromosome 2, with a gene that codifies a protein implicated in photosynthesis, and a no apical meristem protein coding gene with SNP3565318 located in chromosome 5.

4 | DISCUSSION

The root rot caused by *A. euteiches* is a major concern in pea production. Some PR lines have been described, and although their deployment seems to be the most reliable and durable strategy to manage this disease, the complex quantitative nature of this type of resistance hinders its progress in breeding. In this study, we applied a GWAS approach to search for new genomic regions and markers associated with pea foliar and root response to *A. euteiches* and identify candidate genes. For this purpose, a 323 pea accessions collection, not yet explored in this type of genetic study, was foliar and root evaluated 20 days after inoculation with the RB84 strain (pathotype I) under controlled conditions. Subsequently, the resulting phenotypic data were statistically integrated with the genotypic information of the collection, enabling the identification of genomic regions associated with pea resistance to *A. euteiches*.

The evaluated collection exhibited a wide range in foliar and root symptomatology under the tested conditions. In a previous work, the use of the foliar scoring index in combination with the traditional root evaluation to describe the diverse responses of this collection to *A. euteiches* was validated (Rodriguez-Mena et al., 2024). Although that study identified new accessions with meaningful levels of partial resistance, the genetic basis underlying their responses remained unexplored. The present GWAS addressed this gap by identifying SAMs linked with foliar and root response against *A. euteiches*. The location of several of these SAMs in genes known to be involved in plant-pathogen interaction strengthened and served as proof for the usefulness of the used association panel. Additionally, the partial resistance of accessions JI 1428 and JI 1107 was reaffirmed in this work. Nonetheless, all the accessions that exhibited lower symptomatology (RRI $\leq$ 6 and FSI $\leq$ 2) were landraces or wild varieties with undesirable agronomic characteristics, such as the colored flowers of JI 1428 and JI 1107. These accessions represent valuable sources of resistance that should be improved through breeding programs to retain their resistance while eliminating undesirable traits.

Our work allowed the identification of 27 SAMs, each explaining only a small fraction of the phenotypic variance. In combination with the high heritability of both scoring indices and the distribution of the SAMs across the seven pea chromosomes, the polygenic and quantitative nature of resistance to *A. euteiches* in pea was confirmed also in this population (Leprévost et al., 2023; Wu et al., 2018).

Previous studies defined 10 consistent QTLs associated with *A. euteiches* resistance in pea is located one in chromosome 2 (*Ae-Ps1.2*), two in chromosome 3 (*Ae-Ps5.1* and *Ae-Ps5.2*), three in chromosome 4 (*Ae-Ps4.1*, *Ae-Ps4.3*, and *Ae-Ps4.5*), two in chromosome 5 (*Ae-Ps3.1* and *Ae-Ps3.2*), one in chromosome 6 (*Ae-Ps2.2*), and one in chromosome 7 (*Ae-Ps7.6*). *Ae-Ps4.5* and *Ae-Ps7.6* are considered major QTL for pathotypes III and I, respectively, and the other ones as minor QTL (Desgroux et al., 2016; Hamon et al., 2013; Lavaud et al., 2024; Leprévost et al., 2023; Wu et al., 2021). Twelve of the identified SAMs in this study were located in some of these consistent QTLs. Four of them are in the minor effect QTL *Ae-Ps2.2* (DART5914915, DART5898112, DART5922839, and DART5916338) (chromosome 6), and the other seven SAMs distributed across *Ae-Ps1.2* (SNP3564259 and DART3558494) (chromosome 2), *Ae-Ps5.2* (SNP4663348) (chromosome 3), *Ae-Ps4.3* (SNP3554107_2) (chromosome 4), *Ae-Ps4.5* (DART3555273) (chromosome 4), *Ae-Ps3.2* (DART5944388) (chromosome 5) and the major pathotype I QTL *Ae-Ps7.6* (SNP3539262 and DART3561046) (chromosome 7).

In the studied collection, 16 SAMs were identified outside the previously reported consistent QTLs. Among them, a set of closely positioned markers on chromosome 2 (DART4660168, SNP5957817, and DART5955376) and another on chromosome 5 (SNP3565318, SNP5940790, DART3538180, and DART5934328) delineate two putative genomic regions associated with the resistance response. Remarkably, these two regions encompass the common SAMs between both scoring indices and SAMs with a significantly higher frequency of the PR major allele in the PR group with respect to the other groups. These findings suggest that these regions on chromosomes 2 and 5 represent novel loci that play a central role in resistance to *A. euteiches* in the studied population.

Most of the identified SAMs were located within coding sequences, and several of the associated genes belonged to functional categories previously implicated in pea–*A. euteiches* resistance, reinforcing their potential role. Notably, *Psat4g184760*, which encodes a putative lipoxygenase involved in the jasmonic acid biosynthesis pathway, has been consistently highlighted in previous transcriptomic studies as a strong candidate for resistance against *A. euteiches*, further validating its role in the defense mechanism of pea (Kälin et al., 2024; Wu et al., 2022). In addition, other candidate genes encode functions associated with *A. euteiches* pea resistance includes cellulose synthase, leucine-rich repeat receptor, fatty-acid metabolism-involved genes, hydrolases, metabolite transporters, and transcriptional regulators (Al Bari et al., 2023; Desgroux et al., 2016; Hamon et al., 2013; Kälin et al., 2024; Leprévost et al., 2025; Wu et al., 2021, 2022). Beyond these previously established categories, we also identified *Psat5g021760*, an exostosis family gene, which has not yet been associated with *A. euteiches* resistance in pea but has been linked to Fusarium root rot resistance in faba bean, suggesting a potential function in root rot in pea (Webb et al., 2025). Nevertheless, the relevance of these candidate genes in the resistance mechanism requires further functional validation.

In this study, two types of genomics markers, SNP and SilicoDArT, were used. Most of the detected SAMs were SilicoDArT, which could be explained by the higher number of this type of marker in the original dataset. In terms of chromosomal distribution, all SAMs on chromosome 6 were SilicoDArT markers, whereas those on chromosome 1 were exclusively SNPs. Other chromosomes with detected SAMs showed a combination of both marker types. Notably, SNP markers allowed the identification of SAMs that were common to both scoring indices. In this study, the use of both SilicoDArT and SNP markers provided comprehensive coverage of the genome. Combining both types of markers allowed the identification of candidate regions that might have been missed if only one marker type had been employed.

The performance assay was developed under controlled conditions and only with one *A. euteiches* pathotype. Studying the response of this collection in field conditions and with other pathotypes could reinforce the presented results.

Altogether, the present genome-wide association study underscores the quantitative and polygenic nature of resistance to *A. euteiches* in pea. Interesting sources of resistance were identified, and SAMs were located across all seven pea chromosomes, with particular emphasis on loci located within previously reported consistent QTLs on chromosomes 2, 3, 4, 5, 6, and 7. Importantly, the association with the *Psat4g184760* gene, previously related to this resistance, further supports its potential functional relevance. Additionally, SAMs in chromosome 2 and chromosome 5 seem to indicate previously unreported new loci that play a relevant role in *A. euteiches* resistance in the studied population. These findings contribute to a more comprehensive understanding of the genetic basis of Aphanomyces root rot resistance in pea and open new avenues to continue characterizing the mechanism behind this complex trait.

AUTHOR CONTRIBUTIONS

Sara Rodriguez-Mena: Data curation; formal analysis; investigation; methodology; visualization; writing—original draft. **Maria Carlota Vaz Patto**: Conceptualization; resources; supervision; validation; writing—review and editing. **Susana Trindade Leitão**: Methodology; supervision; writing—review and editing. **Diego Rubiales**: Conceptualization; funding acquisition; project administration; writing—review and editing. **Mario González**: Investigation; methodology; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

DArTSeq and SNP datasets analysed in the current study are available in the Zenodo repository, https://zenodo.org/records/7180467.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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