



HAL
open science

Fine mapping of Ae-Ps4.5, a major locus for resistance to pathotype III of *Aphanomyces euteiches* in pea

Clément Lavaud, Angélique Lesné, Théo Leprévost, Marie-Laure Pilet-Nayel

► To cite this version:

Clément Lavaud, Angélique Lesné, Théo Leprévost, Marie-Laure Pilet-Nayel. Fine mapping of Ae-Ps4.5, a major locus for resistance to pathotype III of *Aphanomyces euteiches* in pea. TAG Theoretical and Applied Genetics, 2024, Theoretical and Applied Genetics, 137 (2), pp.47. 10.1007/s00122-024-04548-6 . hal-04483926

HAL Id: hal-04483926

<https://hal.science/hal-04483926v1>

Submitted on 29 May 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Fine mapping of Ae-Ps4.5, a major locus for resistance to pathotype III of *Aphanomyces euteiches* in pea

This Accepted Manuscript (AM) is a PDF file of the manuscript accepted for publication after peer review, when applicable, but does not reflect post-acceptance improvements, or any corrections. Use of this AM is subject to the publisher's embargo period and AM terms of use. Under no circumstances may this AM be shared or distributed under a Creative Commons or other form of open access license, nor may it be reformatted or enhanced, whether by the Author or third parties. By using this AM (for example, by accessing or downloading) you agree to abide by Springer Nature's terms of use for AM versions of subscription articles: <https://www.springernature.com/gp/open-research/policies/accepted-manuscript-terms>

The Version of Record (VOR) of this article, as published and maintained by the publisher, is available online at: <https://doi.org/10.1007/s00122-024-04548-6>. The VOR is the version of the article after copy-editing and typesetting, and connected to open research data, open protocols, and open code where available. Any supplementary information can be found on the journal website, connected to the VOR.

For research integrity purposes it is best practice to cite the published Version of Record (VOR), where available (for example, see ICMJE's guidelines on overlapping publications). Where users do not have access to the VOR, any citation must clearly indicate that the reference is to an Accepted Manuscript (AM) version.

Fine mapping of *Ae-Ps4.5*, a major locus for resistance to pathotype III of *Aphanomyces euteiches* in pea

Clément Lavaud¹, Angélique Lesné¹, Théo Leprévost¹ and Marie-Laure Pilet-Nayel^{1*}

¹ IGEPP, INRAE, Institut Agro, Univ Rennes, 35653 Le Rheu, FRANCE; lavaud.clement@gmail.com (CL); angelique.lesne@inrae.fr (AL); theo.leprevost@inrae.fr (TL); marie-laure.pilet-nayel@inrae.fr (MLPN)

*Correspondance : marie-laure.pilet-nayel@inrae.fr

Key message:

QTL mapping and recombinant screening confirmed the major effect of QTL *Ae-Ps4.5* on pea resistance to pathotype III of *Aphanomyces euteiches* and fine-mapped the QTL to a 3.06 Mb interval.

Abstract:

Aphanomyces root rot, caused by *Aphanomyces euteiches*, is the most-important disease of pea (*Pisum sativum* L.) worldwide. The development of pea resistant varieties is a major challenge to control the disease. Previous linkage studies identified seven main resistance quantitative trait loci (QTL), including the QTL *Ae-Ps4.5* associated with partial resistance in U.S. nurseries infested by the pea pathotype III of *A. euteiches*. This study aimed to confirm the major effect of *Ae-Ps4.5* on *A. euteiches* pathotype III, refine its interval and identify candidate genes underlying the QTL. QTL mapping on an updated genetic map from the Puget x 90-2079 pea recombinant inbred line (RIL) population, identified *Ae-Ps4.5* in a 0.8 cM confidence interval with a high effect ($R^2=89\%$) for resistance to the Ae109 reference strain of *A. euteiches* (pathotype III) under controlled conditions. However, the QTL mapping did not detect *Ae-Ps4.5* for resistance to the RB84 reference strain of *A. euteiches* (pathotype I). Screening 224-pea BC5F2 plant progeny derived from three near-isogenic lines (NILs) carrying the 90-2079 allele at *Ae-Ps4.5* in the Puget genetic background with 26 SNP markers, identified 15 NILs showing recombination in the QTL interval. Phenotyping of the recombinant lines for resistance to the Ae109 strain of *A. euteiches* reduced the QTL to a physical interval of 3.06 Mb, containing 50 putative annotated genes on the Caméor pea genome V1a among which three candidate genes highlighted. This study provides closely-linked SNP markers and putative candidate genes to accelerate pea breeding for resistant varieties to *Aphanomyces* root rot.

Keywords: *Pisum sativum*, *Aphanomyces* root rot, QTL mapping, candidate genes

1. Introduction

Pea (*Pisum sativum* L.) is a legume crop playing an important role for the development of sustainable agricultural systems worldwide. Pea has important agronomic traits due to its ability to fix atmospheric nitrogen through *Rhizobium leguminosarum* symbiosis, offering (i) high protein rich seeds for human or animal consumption, and (ii) environmental benefits in low-input cropping systems. However, pea crops are subjected to various biotic and abiotic stresses causing high yield variability.

Aphanomyces root rot, caused by the oomycete *Aphanomyces euteiches*, is a major disease of pea worldwide (Papavizas and Ayers, 1974). *A. euteiches* has a broad host-spectrum in legumes including lentil (*Lens culinaris*), alfalfa (*Medicago sativa*), common bean (*Phaseolus vulgaris*), clover (*Trifolium repens* and *T. pratense*), vetch (*Vicia sativa*), and faba bean (*Vicia faba*) (Malvick and Percich, 1998; Moussart et al., 2008). The pathogen is able to produce oospores showing a high level of resistance to extreme environmental conditions and an extended life-time (from 10 to 20 years) in soils or root residue (Sherwood and Hagedorn, 1962). The primary symptoms of the disease are necrosis of the roots and softening of the epicotyl, resulting in wilting and chlorosis of plant shoots (Hughes and Grau, 2007). Two main pathotypes of *A. euteiches* are currently reported in pea, based on differential responses of six pea genotypes to infection, including pathotype I detected in several countries around the world and pathotype III which has only been reported in the United States and Canada (Wicker and Rouxel, 2001; Wicker et al., 2003; Le May et al., 2018; Quillévéré-Hamard et al., 2018; Sivachandra Kumar et al., 2021).

Long rotations of pea crops alternating with non-host species is recommended to limit the development of the pathogen in cultivated soils. In addition, predictive tests have been developed to detect and quantify the presence of *A. euteiches* in contaminated soils in order to avoid the cultivation of pea crops (Gibert et al., 2021). However, no highly effective method of control is currently available. Partially resistant germplasm and varieties have been released for the past 30 years, especially in the United States and France (Gritton, 1990; Kraft, 1992; Davis et al., 1995; Roux-Duparque et al., 2004; McGee et al., 2012). But, progress in pea breeding has been difficult due to the (i) polygenic inheritance of the partial resistance to *A. euteiches*, (ii) negative linkages with undesired traits, and (iii) lack of knowledge about the pathogen biology and populations present in infested soils. Thanks to the advanced of molecular and genomic technologies in the past decades, efforts have been made to tackle these issues and better understand the polygenic nature of the quantitative resistance to *A. euteiches*.

Previous quantitative trait locus (QTL) mapping analyses for resistance to Aphanomyces root rot identified seven main “*Ae-Ps*” QTL, named *Ae-Ps1.2*, *Ae-Ps2.2*, *Ae-Ps3.1*, *Ae-Ps4.1*, *Ae-Ps4.5*, *Ae-Ps5.1* and *Ae-Ps7.6*. These QTL

were consistently detected from various environments and isolates, using genetic linkage analysis from four recombinant inbred line (RIL) populations (Hamon et al., 2011; 2013) and/or genome-wide association study (GWAS) from a pea-*Aphanomyces* collection (Desgroux et al., 2016). Resistance alleles at these QTL were derived from four resistance sources, *i.e.* 90-2079, 90-2131 (Kraft, 1992), PI180693 (Lockwood and Ballard, 1960) and 552 (Gritton, 1990), previously identified or registered in the United States. Out of the seven main QTL, *Ae-Ps7.6* displayed a major and consistent effect for resistance in American and French disease nurseries and in controlled conditions using the RB84 reference strain (pathotype I) of *A. euteiches*. Resistance QTL have been successfully introduced in pea breeding programs in France, resulting in the recent registration of tolerant varieties (Moussart, 2022). Quillévéré-Hamard et al. (2021) recently reported that the QTL *Ae-Ps7.6* could display a moderate spectrum of efficacy on French pathogen populations, thus suggesting to combine it with other QTL to increase and preserve the efficacy and durability of the partial resistance. The QTL *Ae-Ps4.5*, previously named *Aph1*, was identified with a major effect in American nurseries (Pilet-Nayel et al., 2002), especially from Minnesota where *A. euteiches* isolates from pathotype III were characterized. The major effect of the QTL was also reported in controlled conditions for the resistance to the reference strain Ae109 (pathotype III) using pea near-isogenic lines (NILs) carrying the 90-2079 allele at the QTL (Lavaud et al., 2015). This QTL could be of high interest in QTL pyramiding strategies to increase the level and broaden the spectrum of partial resistance in pea varieties.

This study aimed to (i) confirm the major effect of QTL *Ae-Ps4.5* against the pea pathotype III of *A. euteiches* and (ii) refine its interval in order to identify closely-linked markers for marker-assisted selection and candidate genes for a better understanding of resistance mechanisms. We performed a QTL mapping analysis for resistance to two reference strains of *A. euteiches* (RB84 and Ae109, pathotypes I and III, respectively) in the Puget x 90-2079 RIL population reported in Pilet-Nayel et al. (2002), using an updated high-density genetic map described in Leprévost et al. (2023). In addition, we conducted a fine mapping analysis of the *Ae-Ps4.5* major QTL by screening and phenotyping recombinant lines derived from NIL progenies segregating in the QTL interval (Lavaud et al., 2015).

2. Materials and Methods

2.1. Plant and fungal materials

A total of 121 pea RILs from the cross Puget (susceptible) x 90-2079 (partially resistant), were used for QTL mapping (Pilet-Nayel et al., 2002; 2005). The two RIL parental lines, Puget and 90-2079, and the two pea lines PI180693 and MN313 (Kraft, 1992; Wicker and Rouxel, 2001), were used as controls in pathogenicity tests.

PI180693 is partially resistant to pathotypes I and III of *A. euteiches* and MN313 is highly resistant to pathotype III.

The pea NIL progeny, consisting of 224 BC5F2 plants segregating at the QTL *Ae-Ps4.5*, was used for QTL fine-mapping. The progeny were developed by self-pollination of three BC5F1 NILs derived from the cross between the donor line RIL831.08, derived from the Puget x 90-2079 RIL population, and the recipient line Puget. NIL production was previously reported in the marker-assisted backcrossing (MAB) program described by Lavaud et al. (2015). The three NILs were heterozygous at the QTL and homozygous for the recipient alleles over the rest of the genome, as confirmed by high-density genotyping data obtained using the GenoPea Infinium® BeadChip (Tayeh et al., 2015) (data not shown). A subsequent population of 59 BC5F4 fixed lines was used for phenotyping test. The two parents of the NIL population, RIL831.08 and Puget, and the source of resistance 90-2079 were used as controls in the NIL pathological test.

The two reference strains RB84 and Ae109 (from pathotypes I and III, respectively), were used for inoculations conducted in a climatic chamber. RB84, isolated from a French infested soil (Riec-sur-Belon, Finistère) (Moussart et al., 2007), was used for inoculation of the RIL population. Ae109, isolated from an American infested soil (Wisconsin, USA) (Malvick et al., 1998), was used to inoculate of RIL and NIL populations.

2.2. Pathological tests

The RILs, NILs and controls were inoculated with *A. euteiches* strains in controlled conditions, using a modified version of the standardized test developed to evaluate *Aphanomyces* resistance in pea (Moussart et al., 2001).

For each strain, all the lines from each RIL or NIL population and control lines were evaluated within one test, in a randomized complete block design with four replicate blocks per test. In each block, five seeds of a single genotype were sown in a pot containing vermiculite. Each isolate × genotype × replicate combination consisted of 20 plants. Plants were grown under constant conditions a photoperiod of 16h at 25 °C and dark period of 8h at 23 °C. Seven days after sowing, seedlings were inoculated (5 ml / plant) using a suspension of zoospores concentrated at 200 spores per ml, from a pure culture of the French or American strain of *A. euteiches*. Zoospores were produced as previously described by Moussart et al. (2001) using an incubation temperature at 25°C and 22°C for RB84 and Ae109, respectively. Capillary watering after inoculation was carried out to favour the disease development and avoid any zoospores leaching in the vermiculite. Seven days after inoculation, Disease Severity (DS) was assessed according to a 0 to 5 scoring scale (0 = no symptom; 5 = dead plant) as described in (Lavaud et al., 2015).

2.3. Genotyping

The genotyping dataset of the Puget × 90–2079 RIL population used for QTL analysis is described in Leprévost et al. (2023). The dataset is comprised of a total of 669 markers, including 504 SNPs, 37 SSRs and 128 other markers (77 AFLP, 41 RAPD, four isozymes, two STS, two SCAR and two morphological markers), and 121 RILs. The Puget x 90-2079 genetic map generated from this dataset is comprised of 669 markers, distributed over 19 linkage groups (LGs), and covering 644.8 cM Haldane.

The NIL population and fixed lines were genotyped i) from DNA extracted from six leaf discs of 6mm, on Limagrain genotyping platform (Limagrain Europe, Clermont-Ferrand, France) and ii) using SNPs designed in KASPTM assays from Duarte et al. (2014), Tayeh et al. (2015) and Boutet et al. (2016).

2.4. Statistical and QTL mapping analysis

Statistical and QTL analysis were performed using R software version 4.0.2 (R Development Core Team, 2020).

For each pathological test, the absence of significant genotype × replicate interactions ($P > 0.05$) was verified using a linear model (John and Sanford, 2019). Then, DS scoring data obtained from RIL or NIL populations and control lines were analysed using a linear mixed model (Bates et al., 2015), including the genotype as the fixed factor, and the replicate and genotype × replicate interaction as random factors. A Wald F test was performed to evaluate significant genotypic effect ($\alpha = 5\%$). Broad sense heritability (h^2) was estimated using the following formula: $h^2 = 1 - (1/F)$, where F is the value of the Fisher test computed from variance analysis in the linear mixed model (Bates et al., 2015). Estimated Marginal Means (EMMs) of DS scores were estimated for each line and from each model (Searle et al., 1980; Lenth, 2023). Pearson correlation analysis was carried out between EMMs obtained with the two *A. euteiches* strains in the RIL population (Harrell and Dupont, 2019). EMMs values from NIL lines were compared between each other using the Tukey multiple comparison test ($\alpha = 5\%$) (Graves et al., 2019).

QTL mapping in the Puget x 90-2079 RIL population was performed from DS scores (EMMs values) obtained in this study for resistance to RB84 and Ae109 strains of *A. euteiches* under controlled conditions. The RIL genetic map and QTL detection method are described in Leprévost et al. (2023). Briefly, composite interval mapping (CIM) was performed using the R/qtl package (Broman et al., 2003). Significant QTL were detected by forward-backward stepwise regression ($\alpha = 5\%$) and validated using a LOD score threshold estimated using 1,000 permutations. QTL interval was defined as the LOD-1 support interval from the peak marker.

2.5. Fine mapping and candidate genes identification

Sequences of markers flanking the QTL *Ae-Ps4.5* were used to identify the physical interval of the QTL genetic region on the Caméor pea genome v1a [JBrowse, URGI <https://urgi.versailles.inra.fr>; (Kreplak et al., 2019)]. Allelic variants between Puget and 90-2079 corresponding to non-synonymous mutations or stop codons generating changes or stops in amino acid sequences, were identified in the annotated genes comprised in the physical interval of the QTL using whole-exome sequencing data produced on the pea Architecture and Multi-Stress (AMS) collection available at URGI, INRAE, France (Aubert et al., 2023).

3. Results

3.1. *Aphanomyces* root rot resistance under controlled conditions

For each strain of *A. euteiches*, analysis of variance (Wald F test) of DS scores obtained in the Puget x 90-2079 RIL population indicated significant genotype ($P < 2.2E-16$) and replicate effects ($P < 6.0E-4$ and $P < 3.4E-15$ for RB84 and Ae109 strains, respectively). Mean-based heritability computed from DS scores were very high with both isolates ($h^2 = 0.98$ and 0.78 with Ae109 and RB84, respectively). Frequency distribution of DS scores (EMM values) obtained with Ae109 and RB84 strains fitted a bimodal and a normal curve, suggesting a monogenic and a polygenic inheritance for these traits, respectively (Fig. 1; Add file 1). Transgressive RILs with increased resistance to each strain were observed (Fig. 1). RB84 and Ae109 DS scoring data were poorly correlated between each other ($r = 0.25$, p -value = 0.006). However, DS scoring data assessed from control lines were consistent with previous studies and confirmed the pathotypes of RB84 and Ae109 strains. MN313 was resistant to the Ae109 strain (EMMs DS = 1.56) but susceptible to RB84 (EMMs DS = 3.29). PI180693 was partially resistant to both strains (EMMs DS = 2.30 and 2.35 for Ae109 and RB84, respectively) (Fig. 1; Add file 1).

3.2. QTL identification

QTL mapping identified four QTL, distributed over three different genomic regions, for resistance to RB84 and Ae109 strains in the Puget x 90-2079 RIL population (Table 1).

Ae-Ps4.5 was identified with a major effect for resistance to the Ae109 strain. The QTL was detected with a LOD score value of 60 and explained 89% of the phenotypic variation. In this region, the allele from 90-2079 contributed to the resistance (Table 1, Fig. 2). The QTL covered 0.8 cM comprising a peak located at 61.8 cM on LG IV.2 of the Puget x 90-2079 genetic map. The QTL detected in this study from the Ae109 strain co-localized with QTL re-detected ($R^2 < 44\%$) from variables scored in US fields (Pilet-Nayel et al., 2002) (Fig. 2). However, *Ae-Ps4.5* was not detected from the RB84 strain data (Table 1).

Ae-Ps5.1 was detected on LGV.1 from RB84 and Ae109 strain data ($R^2 = 16\%$ and 2% , respectively). The resistant parent 90-2079 contributed to the resistance for both strains in this region (Table 1). The QTL interval associated with resistance to RB84 and Ae109 strains covered 3.8cM and 10.6cM, respectively. Both intervals did not overlap between each other but colocalized with three QTL previously detected for *Aphanomyces* root rot resistance from other field (ADI and RRI traits) and greenhouse (Ae106 strain) experiments challenging the Puget x 90-2079 RIL population (Leprévost et al., 2023). In the *Ae-Ps5.1* region, the marker peak identified from the RB84 strain was the “R” locus associated with wrinkled/smooth seed pods (Table 1).

A new QTL was identified from the RB84 strain data at 13.2cM on LG I.2. The QTL explained 12% of the phenotypic variation, covered 7.1 cM, and showed no colocalization with any other QTL detected from variables previously scored in the Puget x 90-2079 RIL population (Leprévost et al., 2023). Resistance at this QTL was from by the Puget allele (Table 1)

3.3. Fine mapping of *Ae-Ps4.5*

The NIL progeny of 224 BC5F2 plants was genotyped using four polymorphic KASP™ SNPs markers (Ps001417, Ps001025, Ps001887 and Ps001281) (Duarte et al., 2014) located in the *Ae-Ps4.5* QTL region. The SNPs covered a large *Ae-Ps4.5* interval of 21.3 cM on the composite map described in Duarte et al. (2014), identified from previous QTL analysis in the RIL population (Pilet-Nayel et al., 2002). Out of the 224 plants, 48 plants showing recombination in the QTL region, as well as 11 plants displaying parental profiles in the QTL interval, were self-pollinated. Each plant was derived from a different mother line, except for two genotypes (FP159 and FP162). The subsequent population of 59 BC5F4 fixed lines, representing seven different BC recombination profiles and the two parental profiles, was phenotyped for resistance to the Ae109 strain of *A. euteiches* (Add file 2).

Analysis of phenotyping data from the 59 BC5F4 lines and three control lines (90-2079, RIL831.08, and Puget) for resistance to the Ae109 strain of *A. euteiches* showed significant genotype x replicate interaction ($P < 2.2E-16$). Analysis of variance identified a highly significant genotype effect ($P < 2.2E-16$). EMMs of DS scores obtained from all the NILs and control lines ranged from 1.52 to 3.42, and averaged 2.72 (Add file 2). Nine NILs showed significantly higher levels of resistance and shared same genotyping profiles as the resistant parent RIL831.08 at markers Ps001887 and Ps001281, separated by a genetic distance of 6.7 cM on the composite maps described in Duarte et al. (2014). A selection of 15 partially resistant or susceptible NILs showing recombination in the interval between markers Ps001887 and Ps001281 were genotyped with 22 additional polymorphic SNPs

(Tayeh et al., 2015; Boutet et al., 2016). The 22 markers were positioned within the interval separating the two markers, except for one marker, *i.e.* Ps112218 (Fig. 3A, Add file 2).

Genotyping profiles of the 15 NILs, obtained using the 22 additional SNPs, were all distinct (Fig. 3B). The Tukey test showed two distinct groups of DS scores (EMM values) among the 15 NILs. Six recombinant NILs (NIL1-4.5_R1 to NIL1-4.5_R6) and the resistance donor parent RIL831.08 had a significant higher level of resistance (average DS value of 1.83) than the nine other recombinant NILs (NIL1-4.5_R7 to NIL1-4.5_R15) and the susceptible parent Puget (average DS value of 3.05) (Fig. 3C; Add file 2). These two groups of NILs shared common genotyping profiles with those of the resistant and susceptible parents, respectively, in a genetic region of 1.5 cM located on the consensus marker map (Leprévost et al., 2023). This fine-mapped region was located between the two markers PsCam039007_23994_241 and PsCam034008_19498_1117 (Fig. 3B) and overlapped the QTL interval of 0.8 cM identified by QTL mapping on the Puget x 90-2079 genetic map.

3.4. Physical region and candidate genes underlying *Ae-Ps4.5*

The fine-mapped 1.5 cM region at QTL *Ae-Ps4.5* corresponds to a physical interval of 3.06 Mb on the pea Caméor genome v1a. This physical interval includes 50 putative protein-encoding genes, including 11 genes with unknown functions (Kreplak et al., 2019). Gene sizes range from 275 to 18,416 bp (Add file 3). Non-synonymous variants between Puget and 90-2079 were found in nineteen of these genes and at 35 different SNP positions (Fig 3D). One stop codon variant was found in the Psat4g152840 gene (Add file 3).

4. Discussion

Ae-Ps4.5, a major locus conferring resistance to the pea pathotype III of *A. euteiches*

QTL mapping analyses confirmed the major effect of *Ae-Ps4.5* ($R^2 = 89\%$) for resistance to the Ae109 strain of *A. euteiches*. However, *Ae-Ps4.5* was not detected from RB84 strain data (pathotype I), suggesting that the QTL effect is specific to the *A. euteiches* pathotype III. The major effect of *Ae-Ps4.5* was previously observed using NILs carrying a resistance allele from the reference parent 90-2079 at the QTL (Lavaud et al., 2015). The Ae109 strain originates from Wisconsin, U.S. (Malvick et al., 1998) where the alfalfa crop has been widespread. Leprévost et al. (2023) recently confirmed the effect of the QTL *Ae-Ps4.5* in two American fields ($R^2 < 44.1\%$ and $R^2 < 15.0\%$ in LeSueur (MN) and Pullman (WA) nurseries, respectively), by updating the QTL analysis in the Puget x 90-2079 RIL population previously reported in Pilet-Nayel et al. (2002). Onfroy et al (Comm. Pers.) characterised both *A. euteiches* pathotypes used in this study. They sampled *A. euteiches* isolates from (i) pathotype III and I in

field nurseries from LeSueur and Pullman, respectively, and (ii) both pathotypes from a field nursery in Mount Vernon (WA). The lower effect of the QTL *Ae-Ps4.5* detected from the field nursery in Pullman (WA) [$R^2 < 15\%$; (Leprévost et al., 2023)] may be due to the predominance of pathotype I isolates in the soil and the absence or low presence of pathotype III isolates.

In Canada, Wu et al. (2021) identified a stable QTL on LG IV (chromosome 4), *AeMRCDIPs-4.1*, conferring partial resistance especially to the Ae-MRDC1 isolate sampled at Morden, Canada (pathotype I). This QTL explained up to 18.5% of the phenotypic variation of the resistance and colocalized with the major *Ae-Ps4.5* QTL (Leprévost et al., 2023). In addition, Sivachandra Kumar et al. (2021) showed the predominance of *A. euteiches* pathotype I in Canadian fields, but identified three isolates belonging to pathotype III from a set of 32 isolates sampled in Saskatoon and Alberta. These studies suggest that *Ae-Ps4.5* may display a minor effect in Canadian fields due to the low frequency of isolates belonging to pathotype III. The *Ae-Ps4.5* QTL may also have a residual resistance effect on pathotype I isolates, as observed for broken major resistance genes in other pathosystems (Mundt, 2018; Singh et al., 2022). A better understanding of the distribution of *A. euteiches* pathotypes in infested fields is required to efficiently and durably deploy the major QTL *Ae-Ps4.5* in pea varieties.

Closely linked SNP markers were identified by linkage analysis in the *Ae-Ps4.5* region

In this study, linkage analysis in a RIL population provided useful SNP markers for pea breeding for resistance to the Ae109 strain. The marker “PsCam036554_21688_1217” was identified at the LOD peak of the QTL *Ae-Ps4.5* at the position 81.5 cM on the consensus marker map described in Leprévost et al. (2023). In addition, fine mapping analysis of the Puget x RIL831.08 recombinant NILs confirmed the position of the QTL on the consensus marker map, with a fine-mapped interval positioned at 81 - 82.5 cM that spans the confidence interval identified by QTL analysis (81.1 - 81.6cM).

By GWAS in the pea-Aphanomyces collection, Leprévost et al. (2023) detected a linkage disequilibrium (LD) block named IV.8, previously reported by Desgroux et al (2016) in the *Ae-Ps4.5* QTL region, as significantly associated with resistance to the Ae109 strain of *A. euteiches*. This LD block comprised the significant marker “PsCam039135_24109_1277” detected in the GWAS and covered a genetic interval ranging from 76.1 to 77.2 cM on the consensus marker map. No colocalization was identified between the LD block IV.8 and the fine mapped interval described in our study. However, the pea lines of the pea-Aphanomyces collection sharing the resistance haplotype at the LD block IV.8 were present in low frequency (8.14%, 14 lines out of 172), suggesting a low accuracy of the GWAS to refine the *Ae-Ps4.5* QTL interval in the haplotype analysis [supplementary table 6 in

Leprévost et al, (2023)]. Thirteen pea lines of the pea-Aphanomyces collection, including 90-2079, MN313 and MN314, were identified as sharing the favourable haplotype carried by the resistant parent RIL831.08 in the fine-mapped *Ae-Ps4.5* region (81 - 82.5 cM). The 13 lines carried also the resistance haplotype detected in the LD block IV.8 (76.1-77.2 cM) in Leprévost et al. (2023) , suggesting that low recombination events occurred between the two genetic intervals in the pea-Aphanomyces collection.

This study confirms that linkage analysis is an efficient method to detect resistance alleles present in low frequency in natural diversity panels. In addition, this study shows that QTL mapping analysis can increase the accuracy of detection of rare alleles, since allele frequencies are more balanced in RIL populations. However, the precision of the fine-mapping may be limited in recombinant populations by the small size of the populations (Korte and Farlow, 2013) and the lack of recombination in some genomic regions. Implementing larger population sizes of RILs and NIL progenies may reduce the size of the fine-mapped QTL interval.

Putative candidate genes were detected in the *Ae-Ps4.5* region

A large physical interval of 3.06Mb underlying the QTL *Ae-Ps4.5* was identified in this study. However, the number of annotated genes was limited to 50 in this region. The Caméor pea genome v1a (3.92 Gb) comprises a total number of 44,756 annotated genes and a high rate of repetitive elements ($n = 2,225,175$), mostly corresponding to retrotransposons (Kreplak et al., 2019).

None of the 50 annotated genes underlying *Ae-Ps4.5* were annotated as nucleotide-binding, leucine-rich repeat receptors (NLR), the most important group of plant disease resistance (R) genes (Kourelis and van der Hoon, 2018). Indeed, the Caméor accession used to assemble the pea genome has been characterized as susceptible to *A. euteiches* (data not shown) and is assumed not to contain R genes conferring resistance to the root rot disease. In this study, we identified non-synonymous and stop codon variants between the Puget and 90-2079 RIL parental genotypes in 19 out of the 50 genes identified, using exome capture sequencing data generated on the pea AMS collection (Aubert et al., 2023). These genes encode a diversity of protein function involved in metabolic and regulatory pathways. Quantitative disease resistance (QDR) genes were reported to be involved in various functions associated with pathogen perception, signal transduction, phytohormone homeostasis, metabolite transport and biosynthesis and epigenetic regulation (Gou et al., 2023). This diversity of functions for QDR genes contrasts with qualitative resistance, which is often conferred by NLR proteins (Gou et al., 2023).

Among the 19 genes, three genes were identified as consistent putative candidates to unravel the molecular mechanisms underlying the *Ae-Ps4.5* major QTL. The first one is a gene coding a DEAD/DEAH box helicase.

This gene showed homology with a functional RNA helicase in *Medicago truncatula*, which interacts with the effector AeSSP1256 of *A. euteiches*, impairing root development of *M. truncatula* and promoting pathogen infection (Camborde et al., 2022). The second gene encodes a C2 domain. This gene (ID : “Psat4g152600”) was one of the eight genes identified by bulked segregant RNA-sequencing in the most stable QTL region *AeMRDC1-Ps4.1/2* recently reported (Wu et al., 2022), which colocalized with the QTL *Ae-Ps4.5* in our study. Plant C2 proteins play an important role in membrane growth, development and targeting, as well as in abiotic and biotic stress adaptations by detecting intracellular Ca²⁺ signals. The third gene encodes a subtilase family protein. We found three non-synonymous variants between Puget and 90-2079 in this gene sequence, which may induce important modification in the translated protein. The large family of subtilases shows a wide spectrum of biological functions, ranging from protein renewal and plant development in interactions with the environment, or the involvement in plant defence responses against several pathogens (Figueiredo et al., 2018).

Eleven genes with unknown function were also detected in the fine-mapped QTL interval. Further improvements of the Caméor reference sequence assembly and annotation, as well as further pea pan-genome sequencing (Yang et al., 2022) comprising genomes from resistant genotypes to *A. euteiches*, will be highly valuable to identify relevant candidate genes underlying the QTL *Ae-Ps4.5*.

5. Conclusion

This study confirmed the major effect of the QTL *Ae-Ps4.5* for resistance to the Ae109 strain of *A. euteiches* belonging to pathotype III. Results provide SNP markers closely linked to the QTL *Ae-Ps4.5*, which will be useful in pea marker-assisted selection programs for the deployment of resistant varieties in fields infested by *A. euteiches* pathotype III. The combination of the major effect QTL with other QTL conferring resistance to *A. euteiches* pathotype I will be relevant to expand the resistance spectrum and preserve the durability of the resistance. This study identifies putative candidate genes in the fine mapped QTL interval, as a first step to better understand the molecular mechanisms underlying resistance to *A. euteiches* in pea.

Acknowledgments

We thank the Greenhouse-Experimental Facilities platform of IGEPP, for its contribution to plant material production and experimentation in controlled conditions. We acknowledge Limagrain Europe, FR, for the genotyping of plant material. We are grateful to Isabelle Glory for her contribution to the plant production, Gilles Boutet for his contribution to marker selection and Rémi Ollivier for his help in the analysis of allelic variants.

Authors' contributions

CL generated the phenotypic and genotypic data of the RIL and NIL recombinant populations, carried out statistical and genetic analyses and drafted the manuscript. AL coordinated and participated to the construction of the NIL recombinant populations and participated in the phenotypic and genotypic data acquisition. TL participated in the QTL detection, the comparative QTL/GWA analyses, and contributed to draft the manuscript. MLPN supervised the study and the draft of the manuscript. All authors approved the final draft of the manuscript.

Funding

This work was supported by the French PEAMAS project, which was supported by the FASO (Fonds d'Action Stratégique des Oléoprotéagineux), managed by SOFIPROTEOL, as well as by the PeaMUST project (ANR-11-BTBR-0002), which received funding from the French Government, managed by the Research National Agency (ANR) under the Investments for the Future.

Availability of data and material Heritability of each trait, genotyping and phenotyping information, linkage information and QTL profiles are available in the main manuscript or as supplementary data.

Declarations

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

- Aubert, G., Kreplak, J., Leveugle, M., Duborjal, H., Klein, A., Boucherot, K., Vieille, E., Chabert-Martinello, M., Cruaud, C., Bourion, V., Lejeune-Hénaut, I., Pilet-Nayel, M.L., Bouchenak-Khelladi, Y., Francillonne, N., Tayeh, N., Pichon, J.P., Rivière, N. and Burstin, J., 2023. SNP discovery by exome capture and resequencing in a pea genetic resource collection. *Peer Community Journal* 3, DOI : 10.24072/pcjournal.332.
- Bates, D., Mächler, M., Bolker, B. and Walker, S., 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67, 1 - 48.
- Boutet, G., Alves-Carvalho, S., Falque, M., Peterlongo, P., Lhuillier, E., Bouchez, O., Lavaud, C., Pilet-Nayel, M.L., Riviere, N. and Baranger, A., 2016. SNP discovery and genetic mapping using Genotyping by Sequencing of whole genome genomic DNA from a pea RIL population. *BMC Genomics* 17, 121.
- Broman, K.W., Wu, H., Sen, S. and Churchill, G.A., 2003. R/qtl: QTL mapping in experimental crosses. *Bioinformatics* 19, 889-890.
- Camborde, L., Kiselev, A., Pel, M.J.C., Le Ru, A., Jauneau, A., Pouzet, C., Dumas, B. and Gaulin, E., 2022. An oomycete effector targets a plant RNA helicase involved in root development and defense. *New Phytologist* 233, 2232-2248.
- Davis, D.W., Fritz, V.A., Pflieger, F.L., Percich, J.A. and Malvick, D.K., 1995. MN 144, MN 313, and MN 314 - Garden pea lines resistant to root-rot caused by *Aphanomyces-euteiches drechs.* *Hortscience* 30, 639-640.
- Desgroux, A., L'Anthoëne, V., Roux-Duparque, M., Rivière, J.P., Aubert, G., Tayeh, N., Moussart, A., Mangin, P., Vetel, P., Piriou, C., McGee, R.J., Coyne, C.J., Burstin, J., Baranger, A., Manzanares-Dauleux, M., Bourion, V. and Pilet-Nayel, M.L., 2016. Genome-wide association mapping of partial resistance to *Aphanomyces euteiches* in pea. *BMC Genomics* 17, 124.

- Duarte, J., Riviere, N., Baranger, A., Aubert, G., Burstin, J., Cornet, L., Lavaud, C., Lejeune-Henaut, I., Martinant, J.P., Pichon, J.P., Pilet-Nayel, M.L. and Boutet, G., 2014. Transcriptome sequencing for high throughput SNP development and genetic mapping in Pea. *BMC Genomics* 15(126).
- Figueiredo, J., Sousa Silva, M. and Figueiredo, A., 2018. Subtilisin-like proteases in plant defence: the past, the present and beyond. *Mol Plant Pathol* 19, 1017-1028.
- Gibert, S., Edel-Hermann, V., Moussa Mcolo, R., Gautheron, E., Michel, J., Bernaud, E., Gautheron, N., Sol, J.-M., Capelle, G., Galland, R., Bardon-Debats, A., Lambert, C. and Steinberg, C., 2021. Risk assessment of *Aphanomyces euteiches* root rot disease: quantification of low inoculum densities in field soils using droplet digital PCR. *European Journal of Plant Pathology* 161, 503-528.
- Gou, M., Balint-Kurti, P., Xu, M. and Yang, Q., 2023. Quantitative disease resistance: Multifaceted players in plant defense. *Journal of Integrative Plant Biology* 65, 594-610.
- Graves, S., Piepho, H.P., Selzer, L. and Dorai-Raj, S., 2019. multcompView: visualizations of paired comparisons. R package version 0.1-8, <http://CRAN.R-project.org/package=multcompView>.
- Gritton, E.T., 1990. Registration of 5 root-rot resistant germplasm lines of processing pea. *Crop Sci.* 30, 1166-1167.
- Hamon, C., Baranger, A., Coyne, C.J., McGee, R.J., Le Goff, I., L'Anthoene, V., Esnault, R., Riviere, J.P., Klein, A., Mangin, P., McPhee, K.E., Roux-Duparque, M., Porter, L., Miteul, H., Lesne, A., Morin, G., Onfroy, C., Moussart, A., Tivoli, B., Delourme, R. and Pilet-Nayel, M.L., 2011. New consistent QTL in pea associated with partial resistance to *Aphanomyces euteiches* in multiple French and American environments. *Theoretical and Applied Genetics* 123, 261-281.
- Hamon, C., Coyne, C.J., McGee, R.J., Lesne, A., Esnault, R., Mangin, P., Herve, M., Le Goff, I., Deniot, G., Roux-Duparque, M., Morin, G., McPhee, K.E., Delourme, R., Baranger, A. and Pilet-Nayel, M.L., 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 Biology* 13, 45.

- Harrell, F.E. and Dupont, C., 2019. Hmisc: Harrell miscellaneous. R Package Version 4.2-0, <https://CRAN.R-project.org/package=Hmisc>.
- Hughes, T.J. and Grau, C.R., 2007. *Aphanomyces* root rot or common root rot of legumes. The Plant Health Instructor DOI: 10.1094/PHI-I-2007-0418-01
- John, F. and Sanford, W., 2019. An {R} Companion to Applied Regression, Third edition ed. Sage Publications, <http://CRAN.R-project.org/package=car>.
- Korte, A. and Farlow, A., 2013. The advantages and limitations of trait analysis with GWAS: a review. *Plant Methods* 9, 29.
- Kourelis, J. and van der Hoorn, R.A.L., 2018. Defended to the nines: 25 years of resistance gene cloning identifies nine mechanisms for R protein function. *Plant Cell* 30, 285-299.
- Kraft, J.M., 1992. Registration of 90-2079, 90-2131, and 90-2322 pea germplasms. *Crop Sci* 32, 1076-1076.
- Kreplak, J., Madoui, M.A., Cápál, P., Novák, P., Labadie, K., Aubert, G., Bayer, P.E., Gali, K.K., Syme, R.A., Main, D., Klein, A., Bérard, A., Vrbová, I., Fournier, C., d'Agata, L., Belser, C., Berrabah, W., Toegelová, H., Milec, Z., Vrána, J., Lee, H., Kougbeadjo, A., Térézol, M., Huneau, C., Turo, C.J., Mohellibi, N., Neumann, P., Falque, M., Gallardo, K., McGee, R., Tar'an, B., Bendahmane, A., Aury, J.M., Batley, J., Le Paslier, M.C., Ellis, N., Warkentin, T.D., Coyne, C.J., Salse, J., Edwards, D., Lichtenzweig, J., Macas, J., Doležel, J., Wincker, P. and Burstin, J., 2019. A reference genome for pea provides insight into legume genome evolution. *Nat Genet* 51, 1411-1422.
- Lavaud, C., Lesné, A., Piriou, C., Le Roy, G., Boutet, G., Moussart, A., Poncet, C., Delourme, R., Baranger, A. and Pilet-Nayel, M.L., 2015. Validation of QTL for resistance to *Aphanomyces euteiches* in different pea genetic backgrounds using near-isogenic lines. *Theoretical and Applied Genetics* 128, 2273-2288.
- Le May, C., Onfroy, C., Moussart, A., Andrivon, D., Baranger, A., Pilet-Nayel, M.L. and Vandemark, G., 2018. Genetic structure of *Aphanomyces euteiches* populations sampled from United States and France pea nurseries. *European Journal of Plant Pathology* 150, 275-286.

- Lenth, R.V., 2023. Estimated Marginal Means, aka Least-Squares Means. R package version 1.8.5, <https://cran.r-project.org/web/packages/emmeans/index.html>.
- Leprévost, T., Boutet, G., Lesné, A., Rivière, J.P., Vetel, P., Glory, I., Miteul, H., Le Rat, A., Dufour, P., Regnault-Kraut, C., Sugio, A., Lavaud, C. and Pilet-Nayel, M.L., 2023. An overview of the diversity of QTL and haplotypes for resistance to aphanomyces root rot in pea (*Pisum sativum*), by integrating QTL, GWAS and new advanced backcross population studies. *Frontiers in Plant Science* 14, 1189289.
- Lockwood, J.L. and Ballard, J.C., 1960. Evaluation of pea introductions for resistance to aphanomyces and fusarium root rots. *Michigan Quarterly Bulletin* 42(4), 704-713.
- Malvick, D.K., Grau, C.R. and Percich, J.A., 1998. Characterization of *Aphanomyces euteiches* strains based on pathogenicity tests and random amplified polymorphic DNA analyses. *Mycol. Res.* 102, 465-475.
- Malvick, D.K. and Percich, J.A., 1998. Genotypic and pathogenic diversity among pea-infecting strains of *Aphanomyces euteiches* from the central and western United States. *Phytopathology* 88, 915-921.
- McGee, R.J., Coyne, C.J., Pilet-Nayel, M.-L., Moussart, A., Tivoli, B., Baranger, A., Hamon, C., Vandemark, G. and McPhee, K., 2012. Registration of pea germplasm lines partially resistant to aphanomyces root rot for breeding fresh or freezer pea and dry pea types. *J Plant Regist* 6, 203.
- Moussart, A., 2022. Management of *Aphanomyces* root of pea in France, 8th International Legume Root Diseases workshop. [https://workshop.inrae.fr/ilrd8/layout/set/print/Submission/Management-of-Aphanomyces-root-rot-of-pea-in-France/\(offset\)/1](https://workshop.inrae.fr/ilrd8/layout/set/print/Submission/Management-of-Aphanomyces-root-rot-of-pea-in-France/(offset)/1).
- Moussart, A., Even, M.N. and Tivoli, 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.

- Moussart, A., Onfroy, C., Lesne, A., Esquibet, M., Grenier, E. and Tivoli, B., 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.
- Moussart, A., Wicker, E., Duparque, M. and Rouxel, F., 2001. Development of an efficient screening test for pea resistance to *Aphanomyces euteiches*, in: AEP (Ed.), 4th Eur Conf Grain Legumes. July 8-12th, Cracow, Poland.
- Mundt, C.C., 2018. Pyramiding for resistance durability: theory and practice. *Phytopathology* 108, 792-802.
- Papavizas, G.C. and Ayers, W.A., 1974. *Aphanomyces* species and their root diseases in pea and sugarbeet. A review. *US Dep Agric Tech Bull* 1485, 158.
- Pilet-Nayel, M.L., Muehlbauer, F.J., McGee, R.J., Kraft, J.M., Baranger, A. and Coyne, C.J., 2002. Quantitative trait loci for partial resistance to *Aphanomyces* root rot in pea. *Theor. Appl. Genet.* 106, 28-39.
- Pilet-Nayel, M.L., Muehlbauer, F.J., McGee, R.J., Kraft, J.M., Baranger, A. and Coyne, C.J., 2005. Consistent quantitative trait loci in pea for partial resistance to *Aphanomyces euteiches* isolates from the United States and France. *Phytopathology* 95, 1287-1293.
- Quillévéré-Hamard, A., Le Roy, G., Lesné, A., Le May, C. and Pilet-Nayel, M.L., 2021. Aggressiveness of diverse french *Aphanomyces euteiches* isolates on pea near isogenic lines differing in resistance quantitative trait loci. *Phytopathology* 111, 695-702.
- Quillévéré-Hamard, A., Le Roy, G., Moussart, A., Baranger, A., Andrivon, D., Pilet-Nayel, M.L. and Le May, C., 2018. Genetic and pathogenicity diversity of *Aphanomyces euteiches* populations from pea-growing regions in France. *Front Plant Sci* 9, 1673.
- R Development Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from <http://www.R-project.org>.

- Roux-Duparque, M., Boitel, C., Decaux, B., Moussart, A., Alamié, J., Pilet-Nayel, M.L. and Muel, F., 2004. Breeding peas for resistance to *Aphanomyces* root rot : current main outputs of three breeding programmes, 5th Eur Conf Grain Legumes. June 7-11th, Dijon, France.
- Searle, S.R., Speed, F.M. and Milliken, G.A., 1980. Population marginal means in the linear model: An alternative to least squares means. *The American Statistician* 34, 216-221.
- Sherwood, R. and Hagedorn, D., 1962. Studies on biology of *Aphanomyces euteiches*. *Phytopathology* 52, 150.
- Singh, H., Kaur, J., Bala, R., Srivastava, P., Sharma, A., Grover, G., Dhillon, G.S., Singh, R.P., Chhuneja, P. and Bains, N.S., 2022. Residual effect of defeated stripe rust resistance genes/QTLs in bread wheat against prevalent pathotypes of *Puccinia striiformis* f. sp. *tritici*. *PLOS ONE* 17, e0266482.
- Sivachandra Kumar, N.T., Caudillo-Ruiz, K.B., Chatterton, S. and Banniza, S., 2021. Characterization of *Aphanomyces euteiches* pathotypes infecting peas in western Canada. *Plant Dis* 105, 4025-4030.
- Tayeh, N., Aluome, C., Falque, M., Jacquin, F., Klein, A., Chauveau, A., Bérard, A., Houtin, H., Rond, C., Kreplak, J., Boucherot, K., Martin, C., Baranger, A., Pilet-Nayel, M.L., Warkentin, T.D., Brunel, D., Marget, P., Le Paslier, M.-C., Aubert, G. and Burstin, J., 2015. The GenoPea Infinium® BeadChip allowed the construction of a high-density highresolution gene-based consensus genetic map in pea and provided an overview on the genome organization. *Plant Journal* 84, 1257–1273.
- Wicker, E., Moussart, A., Duparque, M. and Rouxel, F., 2003. Further contributions to the development of a differential set of pea cultivars (*Pisum sativum*) to investigate the virulence of isolates of *Aphanomyces euteiches*. *Eur. J. Plant Pathol.* 109, 47-60.
- Wicker, E. and 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.

- Wu, L., Fredua-Agyeman, R., Hwang, S.F., Chang, K.F., Conner, R.L., McLaren, D.L. and Strelkov, S.E., 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.
- Wu, L., Fredua-Agyeman, R., Strelkov, S.E., Chang, K.F. and Hwang, S.F., 2022. Identification of novel genes associated with partial resistance to *Aphanomyces* root rot in field pea by BSR-seq analysis. *Int J Mol Sci* 23, 9744.
- Yang, T., Liu, R., Luo, Y., Hu, S., Wang, D., Wang, C., Pandey, M.K., Ge, S., Xu, Q., Li, N., Li, G., Huang, Y., Saxena, R.K., Ji, Y., Li, M., Yan, X., He, Y., Liu, Y., Wang, X., Xiang, C., Varshney, R.K., Ding, H., Gao, S. and Zong, X., 2022. Improved pea reference genome and pan-genome highlight genomic features and evolutionary characteristics. *Nat Genet* 54, 1553-1563.

Accepted manuscript

Figure and Table legends

Fig. 1: Frequency distribution of disease severity scores (EMM values) for resistance to two strains of *A. euteiches*, in the Puget x 90-2079 RIL population. The histogram represents the number of lines in each DS class and the red area under the curve represents the population density. Values of the parental and control lines are shown in black and grey, respectively. n: total number of RILs, m: mean \pm standard deviation of the RIL population, h^2 : heritability estimate. (A) Ae109 and (B) RB84 strain of *A. euteiches*

Fig. 2: QTL identified from the Puget x 90-2079 pea RIL population for resistance to the Ae109 strain of *A. euteiches* under controlled conditions. (A) QTL position in cM Haldane on linkage group IV; genetic map described in Leprévost et al. (2023). (B) LOD curve obtained from QTL analysis using R-QTL; LOD threshold is presented with a green dotted line.

Fig. 3: Fine-mapping of the major QTL *Ae-Ps4.5*, from recombinant pea near-isogenic lines (NILs). (A) Positioning of SNP markers on linkage group IV.2 of the consensus marker map described in Leprévost et al. (2023) except for marker Ps001281 which was repositioned according to NIL recombinants. * the four SNPs (Duarte et al., 2014) used in the recombinant screening experiment. (B) Genotyping profiles of 15 recombinant pea NILs from the cross Puget x RIL831.08 and two parental lines, using a total of 26 SNPs in the QTL interval. In red and blue, genotype from the resistant parent RIL831.08 and the susceptible parent Puget, respectively; in grey and white, heterozygous genotype and missing data, respectively. (C) Disease Severity scores (EMM values) obtained on the 15 NILs and two parents for resistance to the Ae109 strain under controlled conditions, on a 0 (healthy plants) to 5 (dead plants) scoring scale. EMM group(s) are indicated by letter(s), according to the Tukey test ($P < 0.05$). (D) Physical mapping of the fine-mapped QTL to a region of 3.06Mb containing 50 annotated genes on the Caméor pea genome V1a (Kreplak et al., 2019); in red, the 19 annotated genes with non-synonymous mutations or stop codons; * Three putative candidate genes.

Table 1: QTL identified from the Puget x 90-2079 pea RIL population for resistance to the RB84 and Ae109 strains of *A. euteiches* under controlled conditions.

| Scoring trait | Linkage Group | Marker ^a | QTL position | QTL position | Flanking Markers (confidence interval) ^c | QTL confidence | QTL confidence | LOD Threshold ^d | LOD ^e | R ² (%) ^f | Average | Average | Additive effect ^g | Favourable allele ^h |
|---------------|---------------|------------------------|-------------------------------------------------------|-----------------------------------------------|--------------------------------------------------------|----------------------------------------------------------------|--------------------------------------------------------|-------------------------------|------------------|------------------------------------|----------------------|--------------------|---------------------------------|-----------------------------------|
| | | | (cM) - Puget x 90-2079 genetic map ^b | (cM) - consensus markermap ^b | | interval of position (cM) - Puget x 90- 2079 genetic map | interval of position (cM) - consensus marker map | | | | effect of 90-2079 | effect of Puget | | |
| RB84 | I.2 | Ps163643 | 13.2 | 50.8 | E3M4_290 - Ps143400 | 10.7 - 17.8 | 46.5 - 53.4 | 3.44 | 3.81 | 12.0 | 3.46 | 3.30 | -0.08 | Puget |
| Ae109 | IV.2 | PsCam036554_21688_1217 | 61.8 | 81.5 | Ps113489 - PsCam036169_21317_1096 | 61.4 - 62.2 | 81.1 - 81.6 | 3.43 | 59.55 | 88.9 | 1.64 | 3.59 | 0.97 | 90-2079 |
| RB84 | V.1 | R | 34.3 | 31.7 | Ps123535 - PsCam043855_27825_1353 | 33.4 - 37.2 | 30.4 - 35.8 | 3.44 | 4.93 | 15.9 | 3.27 | 3.46 | 0.10 | 90-2079 |
| Ae109 | V.1 | NA | 46.1 | 39.9 | Ps125610 - O09_800a | 40.6 - 51.2 | 38.3 - 42.2 | 3.43 | 5.16 | 2.2 | 2.38 | 2.56 | 0.10 | 90-2079 |

^a Marker at the exact position of the estimated maximum Log of likelihood ratio (LOD) score

^b QTL position in cM Haldane on the Puget x 90-2079 genetic map and on the consensus marker map described in Leprévost et al. (2023)

^c Markers flanking the confidence interval at LOD -1 from the LOD peak

^d LOD threshold estimated after 1000 permutations

^e LOD peak value at the QTL position

^f Percentage of phenotypic variance explained by the QTL

^g Effect of substituting "90-2079" alleles for "Puget" alleles at the QTL. A positive sign indicates that QTL alleles increasing the resistance are contributed by the resistant parent whereas a negative sign means that resistant alleles are brought by the susceptible parent

^h Parent carrying the positive allele at the QTL