Mapping QTL Associated with Partial Resistance to Aphanomyces Root rot in Pea (Pisum Sativum L.) using a 13.2K SNP Array and SSR Markers

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Abstract

Aphanomyces root rot (ARR), caused by *Aphanomyces euteiches* Drechs., is a destructive soilborne disease of field pea (*Pisum Sativum* L.). No completely resistant pea germplasm is available, and current ARR management strategies rely on partial resistance and fungicidal seed treatments. In this study, an F_8 recombinant inbred line (RIL) population of 135 individuals from the cross 'Reward' (susceptible) × '00-2067' (tolerant) was evaluated for reaction to ARR under greenhouse conditions with the *A. euteiches* isolate *Ae-MDCR1* and over 2 years in a field nursery in Morden, Manitoba. Root rot severity, foliar weight, plant vigor and height were used as estimates of tolerance to ARR. Genotyping was conducted with a 13.2K single-nucleotide polymorphism (SNP) array and 222 simple sequence repeat (SSR) markers. Statistical analyses of the phenotypic data indicated significant (P<0.001) genotypic effects and significant G×E interactions (P<0.05) in all experiments. After filtering, 3050 (23.1%) of the SNP and 30 (13.5%) of the SSR markers were retained for linkage analysis, which distributed 2999 (2978 SNP + 21 SSR) of the markers onto nine linkage groups representing the seven chromosomes of pea. Mapping of quantitative trait loci (QTL) identified 5 major-effect (R^2 > 20%), 13 moderate-effect (10%<R^2< 20%) effect and 10 minor-effect (R^2 <10%) QTL. A genomic region on chromosome IV, delimited by the SNP markers PsCam037549_22628_1642 and PsCam026054_14999_2864, was identified as the most consistent region responsible for partial resistance to *A. euteiches* isolate *Ae-MDCR1*. Other genomic regions important for resistance were of the order chromosome III, II and VII.

Key Message

A stable and major QTL, which mapped to an approximately 20.0 cM region on pea chromosome IV, was identified as the most consistent region conferring partial resistance to *Aphanomyces euteiches.*

Introduction

Field pea or "dry pea" (*Pisum sativum* L.) is an economically important cool-season legume crop that is cultivated widely in different parts of the world (Hossain et al. 2012). Pea seeds contain 15.8-32.1% total protein, are rich in carbohydrates, calcium, iron, phosphorus and contain various vitamins (Zhang et al. 1985; Burstin et al. 2007; Yoshida et al. 2007; Trinidad et al. 2010), and hence serve as a nutrient-rich food and feed source. Canada produces the most pea worldwide, with about 31% of the market share, followed by Europe (30%), Russia (13%), and China (12%) (FAOSTAT 2017).

Unfortunately, the production of field pea is affected adversely by the pea root rot complex (PRRC) (Bailey et al. 2003; Xue 2003; Chang et al. 2013; Wu et al. 2018). The soilborne oomycete *Aphanomyces euteiches* Drechs. is a dominant pathogen in the PRRC (Jones and Drechsler 1925). This pathogen is favoured by saturated soil conditions and poor drainage. The oospores can survive in the soil for up to 10 years (Papavizas and Ayers 1974; Holliday 1980). Under conducive environmental conditions, yield losses in pea as high as up to 86% can occur in fields infested with *A. euteiches* (Pfender and Hagedorn 1983). In Canada, Aphanomyces root rot (ARR) outbreaks have been reported only recently, either because pea production in the same fields over multiple years resulted in a buildup of the pathogen, or because symptoms of ARR can now be better distinguished from other root rot pathogens (Hwang and Chang 1989; Xue 2003; Chatterton et al. 2015; Wu et al. 2017). Aphanomyces root rot is characterized by the formation of soft and water-soaked rootlets with a honey-brown or blackish-brown color. Reductions in seedling emergence and seedling blight have also been reported. As infected plants continue to grow, secondary infection causes development of brown lesions and cortical decay of the belowground tissues. The uptake of water and nutrients in diseased plants also is reduced, which can result in wilting and death of the plants (Chatterton et al. 2015).

Chemical strategies appear to be of limited value in the control of ARR, due to a lack of effective commercial fungicides (Pilet-Nayel et al. 2002; Wu et al. 2019). The seed treatment INTEGO™ Solo, containing the chemical ethaboxam (Valent Canada, Inc. Guelph, Ontario), is the only product registered in Canada found to suppress the growth of *A. euteiches*. Cultural disease management methods, such as longer rotations with non-host crops and the avoidance of infested fields, have had some success but are not always practical (Malvick et al. 1994; Conner et al. 2013). Genetic resistance could be the most promising approach to control ARR. However, pea cultivars completely resistant to ARR are not available (Pfender et al. 2001; Conner et al. 2013; Gossen et al. 2016). As a result, genotypes with only partial polygenic resistance are being used for the economic and durable control of ARR (Palloix et al. 2009; Kou and Wang 2010; Desgroux et al. 2016; Lavaud et al. 2016). Shehata et al. (1983) identified tolerance to ARR in some plant introduction (PI) lines of pea.

Previous studies to map quantitative trait loci (QTL) utilized morphological traits (e.g., leaf morphogenesis, hilum color on seeds and anthocyanin production), isozymes, and amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), inter-simple sequence repeat (ISSR) and sequence-tagged site (STS) markers (Pilet-Nayel et al. 2002, 2005; Hamon et al. 2011, 2013; Lavaud et al. 2015). The number of PCR-based markers used for genotyping in these studies has ranged from 150-350. As such, the confidence interval for the detected QTL is often very large and the identified QTL usually contain a small number of markers. The use of high-density SNP arrays or next generation sequencing technology (NGS) in peas has only been reported recently (Sindhu et al. 2014; Tayeh et al. 2015; Desgroux et al. 2016; Gali et al. 2018). Reducing the confidence interval in the QTL regions will be required to identify associated markers for marker-assisted selection (MAS).

Pilet-Nayel et al. (2002, 2005) identified one major QTL *Aph1* and three minor QTL *Aph9*, *Aph10* and *Aph11* located on pea chromosome IV to be associated with resistance to ARR in a recombinant inbred line (RIL) population. Additionally, eight minor QTL, *Aph2* located on chromosome V, *Aph3, Aph4* and *Aph5* on chromosome I, *Aph8* on chromosome III, *Aph12* on chromosome VI, and *Aph6* and *Aph13* on chromosome VII, were found to be associated with the resistance to ARR in the same RIL population (Pilet-Nayel et al. (2002, 2005). The major QTL (*Aph1*) accounted for up to 47% of the variability in partial resistance. Hamon et al. (2011, 2013) identified five highly stable QTL associated with partial resistance to ARR in pea. The QTL *Ae-Ps7.6* located on chromosome VII had a major effect on resistance and explained up to 42.2% of the phenotypic variation in 32 of 37 disease variables in two RIL populations. Four other QTL, namely *Ae-Ps1.2* on chromosome I, *Ae-Ps2.2* on chromosome II, *Ae-Ps3.1* on chromosome III, and *Ae-Ps4.1* on chromosome IV, accounted for up to 14.4, 26.9, 29.9 and 24.5 % of the phenotypic variation, respectively, in 13, 22, 11 and 14 of 37 disease variables in the same two RIL populations. Lavaud et al. (2015) identified *Ae-Ps7.*6 and *Ae-Ps4.5*, located on chromosomes VII and IV, respectively, as major-effect QTL for tolerance to ARR in near isogenic lines (NIL) of pea

with different genetic backgrounds. The QTL *Ae-Ps5.1*, located on chromosome V, contributed the least to the resistance in the NILs (Lavaud et al. 2015). Desgroux et al. (2016) identified 52 QTL (with small-size intervals on all seven chromosomes) in a genome wide association study (GWAS) of 175 pea lines. Collectively, these studies suggest that the major QTL for tolerance to ARR in pea are located on chromosomes IV and VII, with several minor QTL on chromosomes I, II, III and V (Pilet-Nayel et al. 2002, 2005; Hamon et al. 2011, 2013; Lavaud et al. 2015; Desgroux et al. 2016).

The purpose of this study was to identify QTL for partial resistance to ARR using high- density SNP markers and SSR anchor markers in an F_8 RIL population of pea derived from the cross 'Reward' (susceptible) ×'00-2067' (tolerant). In addition, QTL for two disease-related traits (foliar weight and vigor) and one agronomic trait (plant height) were evaluated. Lastly, the stability of the genetic loci controlling these four traits was examined under field and greenhouse conditions.

Materials And Methods

Plant Materials

One hundred and thirty-five F_8-derived recombinant inbred lines (RIL) were developed by single-seed descent from the cross '00-2067' × 'Reward'. The parental cultivar '00-2067', was developed by Dr. J. Kraft and V. A. Coffman at the Irrigated Agriculture Research and Extension Center in Prosser, WA, from the crosses (PH14-119×DL-1)7 × (B563-429-2 × PI 257593) × DSP-TAC (Conner et al. 2013). The parental cultivar '00-2067' was reported to be tolerant to ARR, produced wrinkled seed coat, white flowers and was semi-leafless (Conner et al. 2013). The susceptible parent 'Reward' was derived from the cross '4-0359.016' × 'MP1491' and produced white flowers and yellow cotyledons (Bing et al. 2006). The parents '00-2067' and 'Reward' were included in the inoculation experiments as negative and positive controls, respectively.

Fungal isolate

The *A. euteiches* isolate *Ae-MRDC1*, obtained from soil collected from an AAR disease nursery at the Agriculture and Agri-Food Canada (AAFC) Morden Research and Development Centre (MRDC), Morden, MB (lat. 49°11' N, long. 98°5' W), was used in the greenhouse experiments. This isolate was identified as virulence type I following Wicker and Rouxel (2001). Oospore inoculum was generated in an oat broth as described by Papavizas and Ayers (1974); the concentration of oospores was estimated with a haemocytometer and adjusted to a final concentration of 1×10^5 oospores mL^{-1} with sterile deionized water.

Phenotyping under field conditions

Field evaluation of the RILs was conducted in the naturally infested ARR disease nursery at the AAFC MRDC in 2015 and 2016. Pea genotypes planted in this nursery, which is situated on a loamy clay soil, have consistently developed severe ARR symptoms over many years, confirming strong disease pressure (Corner et al. 2013). Field layouts of the 135 RIL and three repeats of the two parental checks, '00-2067' and 'Reward', were generated as generalized lattice designs with the experimental design software CycDesigN® (VSNi, 2015). The layout differed slightly between 2015 and 2016. In 2015, each replicate consisted of nine rows by 16 plots, with a check in each row and the two checks occurring once in each set of three rows. In 2016, the lattice layout was Latinized to account for any gradients from left to right, and up and down the field. The three replicates each with six rows were stacked up the field, and each row contained 24 plots formed by three blocks of eight plots (i.e., 6 rows × 3 blocks × 8 plots/replication). Two blocks each with 48 plots from each of the three replicates created three super-blocks. Each super-block contained 135 RILs and 3 repeats of the two checks.

Plots with 15 seeds/row and 30 seeds/row were sown on May 7, 2015 and May 9, 2016, respectively. Fertilizer applications and weed control were based on standard recommendations for field pea production in the region (Saskatchewan Pulse Growers 2000). Ten plants were uprooted from each plot for trait measurements. Root rot disease severity (DSF) and vigor (VF) were measured on July 22, 2015, and July 19, 2016, with DSF scored on a 0 to 9 scale and VF scored on a 0 to 4 scale following Conner et al. (2013). The foliar weight (Fwt) of the plants evaluated for DSF and VF was obtained after oven drying at 30°C for 10 days.

Phenotyping under greenhouse conditions

Eight seeds of each RIL and both parents were germinated on moistened Whatman No. 1 filter paper in Petri dishes, with the seedlings transplanted into 7 cm × 7 cm × 10 cm plastic pots (1 plant/pot) filled with a sterilized potting mixture (Cell-TechTM, Monsanto, Winnipeg, MB) after 5-days. Briefly, a 5-cm deep-hole was made in the soil in each pot, with one seedling placed in the hole and inoculated with a 1 mL aliquot of the oospore suspension prepared as described above. The rootlets of the inoculated seedlings were then covered with the potting mix, and the plants were placed in a greenhouse with a 12 h photoperiod at day and night temperatures of 22-28°C and 15-18°C, respectively. The pots were arranged in a randomized complete block design (RCBD). Plants were watered daily in the morning and evening to ensure high moisture levels in the potting mixture. The entire inoculation experiment was run independently a total of three times, referred to as greenhouse experiment 1 (GH1), 2 (GH2) and 3 (GH3).

Plant height (HGH) was measured from the top leaf to the soil line at the end of second week after inoculation. At four weeks, the plants were carefully uprooted, washed under standing water, and assessed for disease severity (DSGH) and dried foliage weight (DFGH) as described for the field study.

Statistical analysis of phenotypic data

All of the variables from the field and greenhouse trials were analyzed by station-year or single greenhouse experiment and pooled conditions (i.e., year × location) using R software (R core team 2019). The model for single environment analysis was: $Pij = \mu + Gi + Rj + eij$, where Pij was the score of the i^{th} RIL located in the j^{th} replicate, μ the mean of all the data in a single site-year or greenhouse experiment, Gi the i^{th} RIL effect or genetic effect, Rj the j^{th} replicate effect or blocking effect, and eij was the residual variance. The model for multiple station-years or greenhouse experiments was: $Pijk = \mu + Gi + Rj + Lk + GLijk$

+ eijk, where Pij was the score of the i^{th} RIL located in the j^{th} replicate, μ the mean of all pooled data, Gi the i^{th} RIL effect or genetic effect, Rj the j^{th} replicate effect or blocking effect, Lk the k^{th} environment or location effect, GLijk the interaction of RIL effect and environment, and eijk was the residual variance. The entry-based broad-sense heritability ($H^2=\sigma^2_G/\sigma^2_P$) was calculated as $h^2 = \sigma^2_G/[\sigma^2_G + (\sigma^2_e/r)]$ for single site-years or greenhouse experiments and $h^2 = \sigma^2_G/[\sigma^2_G + (\sigma^2_{GE}/k) + (\sigma^2_e/rk)]$ for pooled conditions, respectively, for which σ^2_G is the genetic variance, σ^2_{GE} is the genotype × environment interaction variance, σ^2_e the residual variance, k the number of environments and r the number of replicates per line. The least square means (LSM) for single site-years or greenhouse experiments and the pooled data of both the field and greenhouse experiments were estimated in R (package 'lsmenas') and histograms of frequency distribution using LSM for pooled data also were made generated with R software. The Pearson correlation coefficient of the LSM was calculated for each variable in the different single site-years or greenhouse experiments as well as in the pooled data. Correlation analysis of the Pearson correlation coefficient among variables also was used to evaluate the relationship between disease tolerance and the agronomic variables. The Shapiro-Wilk test was used to test for normality in the phenotypic data.

Genotyping with SNP and SSR markers

The 135 RILs and the parents were first genotyped using 13,204 high-quality SNP markers selected from a 248,617 SNP marker set developed by Tayeh et al. (2015). The 13,204 SNPs were all derived from gene-encoding sequences and were well distributed across the pea genome (Tayeh et al. 2015). Filtering was conducted to remove failed SNP reactions, markers lacking polymorphism in the parents, low coverage site markers, markers with MAF ≤ 0.05, markers missing data for > 5% of the accessions, and those with segregation distortion.

Two hundred and twenty-two microsatellite markers, reported by Loridon et al. (2005) to be well distributed along the seven linkage groups of pea, were also used to genotype the 135 RILs and the parents. PCR assays were carried out in a 12 μL reaction mixture containing 20 ng of genomic DNA, 1× Taq buffer, 2.0 mM $MgCl_2$, 200 μM dNTPs, 0.4 μM forward primer modified at the 5'-end with an M13 tail, 0.4 μM reverse primer, 0.2 μM fluorescently labeled M13 primer and 1.25 U *Taq* polymerase (Promega, Madison, USA). Amplifications were carried out in a Mycycler Thermal Cycler (Bio-Rad, Mississauga, ON, Canada) with 35 cycles of denaturation at 94°C for 30 s (5 min for the first cycle), annealing for 45 s at a temperature based on the primers used, and extension at 72°C for 1 min.

An aliquot of the PCR products was separated by capillary electrophoresis on an ABI PRISM 3730xl DNA analyzer (Applied Biosystems, Foster city, CA). In addition, the amplified products of the polymorphic markers were separated by electrophoresis on 8% polyacrylamide gels (PAGE) at 150 V for 2 h. The amplified fragments were stained with silver nitrate and photographed with UV Transilluminator (Bio-Rad Laboratories, Mississauga, ON, Canada).

Linkage map construction

A linkage map was constructed with the filtered SNP and the polymorphic SSR markers following the two-step mapping strategy of Perez-Lara et al. (2016). In brief, a draft linkage map was generated using the minimum spanning tree map (MSTMap) software (Wu et al. 2008) with a strict cut off p-value of $1E^{-10}$ and a maximum distance between markers of 15 cM. Only one of multiple markers in the same positions was retained for linkage analysis. The draft map was then refined using MAPMAKER/EXP 3.0 (Lincoln et al. 1992) with a logarithm of odds (LOD) score ≥ 3.0 and recombination fraction (Θ) value ≤ 0.40. The Kosambi map function (Kosambi 1944) was used to calculate the genetic distances (in cM) between the markers. The linkage groups were assigned to chromosomes based on the consensus SNP map of pea developed by Tayeh et al. (2015). Genetic linkage maps were constructed with MapChart v. 2.32 (Voorrips, 2002).

Additive-effect QTL Analysis

Quantitative trait loci detection was carried out over two field seasons (2015 and 2016), three greenhouse experiments and using pooled data for root rot severity (DSF15, DSF16, DSGH1, DSGH2, DSGH3, DSFC and DSGHC), dry foliar weight (DFF15, DFF16, DFGH1, DFGH2, DFGH3, DFFC and DFGHC) and vigor (VF15, VF16, VGH1, VGH2, VGH3, VFC and VGHC). In the case of plant height, QTL detection were conducted on only the three greenhouse and pooled experiments (HGH1, HGH2, HGH3 and HGHC).

Additive-effect QTL were detected by Composite Interval Mapping (CIM) using WinQTL Cartographer v2.5 with the following parameters: 1 cM walking speed, forward and backward regression method, window size 10 cM, five background cofactors, 1000 permutations and $P < 0.05$ (Wang et al., 2011). The QTL positions were identified at regions where the LOD score reached values ≥ 3.0. The confidence interval of each QTL was defined by the consensus region bordered by the five environments (two field and three greenhouse experiments).

Additive-effect QTL were named with the abbreviation "*Ps*" (*Pisum sativum*) followed by the trait (*AeMRDC1*, *Aphanomyces euteiches* isolate *Ae-MRDC1*; *Fwt*, dry foliar weight; *Vig*, Vigor and *Hgt*, height), a hyphen (-), chromosome (I-VII), and the serial number of the QTL (e.g. *Ps.AeMRDC1-C4.1*). The QTL were classified as stable if they were confirmed in at least two of five environments. The percentage of phenotypic variation explained (PVE) due to a particular QTL was estimated by the coefficient of determination (R^2). Furthermore, QTL with R^2 >20%, 10-20% and <10% were arbitrarily classified as major, moderate or minor-effect QTL, respectively.

The origins of favourable alleles for individual traits were assigned to different parents following Lubberstedt et al. (1997) and Zaidi et al. (2015). Alleles coming from '00-2067' were coded as "2" whiles alleles from 'Reward' were coded as '0". The additive effects of each QTL were calculated by deducting the phenotypic average of all individuals carrying the "0" allele from that of individuals with the "2" allele. A negative sign of the additive effect for root rot severity indicates that the favorable allele for the traits originated from the parent '00-2067', while a positive sign indicates it originated from 'Reward'. In contrast, a positive sign of the additive effect for foliar weight, vigor and plant height indicates that the favorable allele for these traits originated from '00-2067', while a negative sign indicated that the favorable allele originated from 'Reward'.

Epistatic-effect QTL analysis

Epistatic-effect QTL (QTL × QTL) were detected with IciMapping V.4.1 using the ICIM-EPI method (Meng et al., 2015). The mapping parameters were the same as those used for the CIM above. Epistatic-effect QTL were named with the abbreviation "*PsE*" followed by the trait and the serial number of the QTL (e.g. *PsE.AeMRDC1-1*, *PsE.Fwt-1*, *PsE.Vig-1* and *PsE.Hgt-1*). The significance R^2 thresholds selected to classify epistatic-effect QTLs as major, moderate or minor were arbitrarily set at R^2 >15%, 7.5-15% and <7.5%, respectively.

Results

Phenotypic trait analysis

The evaluation and frequency distribution of the 135 RILs for disease severity, foliar weight, vigor and plant height for each of the two field seasons and three greenhouse experiments, as well as for the pooled data, are presented in Table 1 and Figure 1, respectively.

Disease severity variation in the RIL population

The parent cultivar '00-2067' was tolerant to *A. euteiches* isolate *Ae-MRDC1*, with an estimated mean DSI of 2.3 ± 1.0 SE, 1.5± 1.1 SE and 1.7 ± 0.6 SE for the three greenhouse experiments (DSGH1, DSGH2 and DSGH3), respectively. The same cultivar was found to be moderately susceptible in the 2015 (DSF15) and 2016 (DSF16) field trials at Morden, MB, with estimated means of 6.1 ± 0.4 SE and 5.9 ± 0.6 SE, respectively. In contrast, the parent 'Reward' was susceptible to ARR, with estimated means of 5.0 ± 1.3 SE, 6.1 ± 0.9 SE and 5.1 ± 0.5 SE for DSGH1, DSGH2 and DSGH3, and 8.1 ± 0.5 SE and 7.6 ± 1.1 SE for DSF15 and DSF16. ANOVA of each field and greenhouse (GH) test showed a significant effect of genotype on the RIL population ($P < 0.05$). The interaction of RIL genotype × year for root rot severity in the disease nurseries was not significant, while there was a significant interaction ($P < 0.05$) of RIL genotype × site under greenhouse conditions. The correlation among individual experiments in the greenhouse and field was significant (r = 0.32-0.83, $P<0.001$). Therefore, the data were pooled in the multiple model. The least square means (LSM) of '00-2067' was calculated as 1.8 ± 0.9 SE for the pooled greenhouse data (DSGHC) and as 6.0 ± 0.5 SE for the pooled field (DSFC) data. In the case of 'Reward', the LSM were 5.3 ± 1.8 SE in the greenhouse and 7.9 ± 0.9 SE in the field. Based on a t-test analysis, the differences in disease severity between the two parental genotypes were significant in both the greenhouse and field experiments ($P < 0.05$). The frequency distributions of the estimated means of the disease severity in the greenhouse and field were continuous (Figure. 1a), with low values for Skewness and Kurtosis (Table 1). The Shapiro-Wilk test of the disease severity data for normality were significant for both the greenhouse and field experiments, and hence the data did not follow a normal distribution. The genotypic effects of disease severity were significant ($P<0.05$) in the greenhouse and field for the individual experiments and the pooled data. Entry mean-based heritability of disease severity was high, ranging from 59% to 92% (Supplementary Table S1), which indicated a strong genetic effect of tolerance to *A. euteiches* that was transmitted from '00267' to individuals in RIL population.

Foliar weight variation in the RIL population

Foliar weight for the parental cultivar '00-2067' had an estimated mean of 3.0 ± 0.7 SE, 2.6 ± 0.5 SE and 2.8 ± 0.5 in the three greenhouse experiments (DFGH1, DFGH2 and DFGH3), respectively, and 15.9 ± 11.7 SE and 5.6 ± 2.1 SE in the 2015 (DFF15) and 2016 (DFF16) field trials. In the case of 'Reward', the estimated means were 2.3 ± 0.8 SE, 1.8 ± 0.8 SE and 2.4 ± 0.5 for DFGH1, DFGH2 and DFGH3, respectively, and 2.7 ± 1.2 SE and 1.1 ± 0.5 SE for DFF15 and DFF16. The pooled data for '00-2067' and 'Reward' were 2.8 ± 0.6 SE and 2.3 ± 0.9 SE for the greenhouse experiments (DFGHC), and 10.8 ± 9.7 SE and 1.9 ± 1.2 SE for the field trials (DFFC), respectively. Significant differences between the parental cultivars were found with respect to foliar weight in the field and greenhouse experiments ($P<0.05$) and in the pooled data. In addition, significant RIL genotype effects were found in the ANOVA of foliar weight in both the greenhouse and field experiment ($P < 0.05$). Foliar weight of the RIL population in both the greenhouse and field trials had a continuous frequency distribution (Figure 1b). However, the data did not follow a normal distribution based on the Shapiro-Wilk test; also, the distribution of DFFC was skewed (Table 1). Foliar weight in the replicated greenhouse and field experiments was correlated significantly (r = 0.44-0.93, $P<0.01$). Heritability of foliar weight was high, ranging from 67% to 94% (Supplementary Table S1). This suggested that a significantly high percentage of genotypic effect ($P<0.05$) was transmitted from the parents to the RIL population.

Vigor variation in the RIL population

The tolerant parent ('00-2067') looked bigger and grew much better than the susceptible parent ('Reward'). This was reflected in the estimated means and least square means for the individual greenhouse and field studies (VGH1, VGH2, VGH3, VF15, and VF16), which were 3.2 ± 0.4 SE, 3.9 ± 0.2 SE, 3.9 ± 0.2 SE, 3.7 ± 0.4 SE, 3.7 ± 0.5 SE, respectively, for '00-2067' vs. 2.5 ± 0.5 SE, 2.3 ± 0.9 SE, 2.5 ± 0.5 SE, 1.9 ± 0.3 SE, 1.6 ± 0.7 SE for 'Reward'. The mean vigor scores for '00-2067' was a significantly higher compared to 'Reward' ($P < 0.05$). A significant genetic variance in the RIL population was detected in the ANOVA ($P < 0.05$). The frequency distribution for vigor in the RIL population for the pooled greenhouse (VGHC) and field data (VFC) were continuous, but only VGHC was normally distributed (Figure. 1c). The individual replications in the greenhouse and field were coincident based on the correlation analysis (r = 0.31-0.88, $P<0.05$). Significant genotypic effect ($P<0.05$) and high heritability (64-90%) were detected in the RIL population (Supplementary Table S1). The pooled data for vigor ratings for '00-2067' were 3.7 ± 0.5 SE and 3.7 ± 0.4 SE in field and greenhouse, respectively, while for 'Reward', the LSMs were 1.7 ± 0.6 SE and 2.5 ± 0.7 SE, respectively.

Plant height variation in the RIL population

Differences in plant height between the parents '00-2067' and 'Reward') were significant ($P < 0.05$) in the pooled data and greenhouse experiment 1 (HGH1). The estimated means in plant heights for '00-2067' were 223.6 ± 13.8 SE, 173.0 ± 25.6 SE and 164.2 ± 30.5 for HGH1, HGH2 and HGH3, respectively, while the estimated means for 'Reward' were 179.5 ± 28.7 SE, 140.3 ± 36.4 SE and 157.6 ± 23.1 for HGH1, HGH2 and HGH3, respectively. The pooled plant height

(HGHC) data for '00-2067' was 187.3 ± 35.4 SE, and for 'Reward' it was 159.3 ± 32.9 SE. Significant RIL genotype effects were detected by ANOVA ($P < 0.05$) in all three greenhouse experiments. Frequency distribution of height in each greenhouse experiment was not normal based on the Shapiro-Wilk test (Figure 1d). The height of both of the parents '00-2067' and 'Reward' was lower than the mean of the RIL population. The heritability of height was 93%, 91%, 92% and 93% for HGH1, HGH2, HGH3 and HGHC, respectively. (Supplementary Table S1).

Correlation analysis among traits

All of the traits in different environments were significantly and positively correlated among each other ($0.31 < r < 0.93$, $P<0.001$). Correlation analysis among variables indicated that all the traits were significantly correlated with each other in the individual experiments and in the pooled data ($0.36 < r < 0.93$, $P<0.001$), except for plant height, which was only coincidently correlated with vigor ($0.20 < r < 0.37$, $P<0.05$) and dry foliar weight ($0.57 < r < 0.68$, $P<0.001$). Root rot severity was negatively correlated with dry foliar weight and plant vigor in both the greenhouse and field experiments. This suggested that ARR had an adverse effect on plant growth.

Genetic linkage mapping

Filtering removed 10,154 (76.9%) of the SNP markers and 192 (86.5%) of the SSR markers used to genotype the 135 RIL population. Therefore, 3050 (23.1%) of the SNP and 30 (13.5%) of the SSR markers used for genotyping were retained for linkage analysis. The linkage analysis distributed 2999 (2978 SNP + 21 SSR) markers on nine linkage groups, while the remaining 81 (72 SNP + 9 SSR) markers were unlinked. The nine linkage groups represented all seven chromosomes of the pea genome (Table 2). The length of the nine linkage groups ranged from 21.1 cM (linkage group 2) to 395.7 cM (linkage group 4). Linkage groups 1 and 2 corresponded to chromosome I (Ia and Ib) of the pea genome, while linkage groups 3, 4, 5, 8 and 9 corresponded to chromosomes II, III, IV, VI, and VII, respectively. Linkage group 6 and 7 corresponded to chromosome V (Va and Vb) of the pea genome. The number of markers per chromosome ranged from 103 (chromosome VI) to 334 (chromosome VII). The length of the seven chromosomes ranged from 155.6 cM (chromosome VI) to 395.7 cM (chromosome III) and spanned a total length of 1704.1 cM. The marker density per loci ranged from 1.1 to 2.4 and averaged 1.8 markers per loci (Table 2).

Additive-effect QTL analysis

One thousand five hundred and seventy-seven (1577) of the 2999 markers (Supplementary Table S2) mapped to the same position as other markers and were excluded from the QTL analysis. Therefore, only 1422 (10.5%) of the initial 13426 (13204 SNP + 222 SS) markers used to genotype the 135 RIL population were used for QTL analysis. Overall, 28 QTL related to tolerance to ARR, foliar weight, vigor and plant height were detected by the CIM method using WinQTL Cartographer v2.5 (Wang et al. 2011). Based on the R^2 values, 10 of the QTL were major-effect QTL, 13 were moderate-effect QTL and 10 were minor-effect QTL. Eight of the 28 QTL were detected consistently in the greenhouse and field experiments, and could be classified as stable, while the remaining 20 were year- or experiment-specific QTL detected in only one environment (pooled data not counted).

QTL for tolerance to ARR

Six putative QTL for partial resistance or tolerance to *A. euteiches* isolate *Ae-MRDC1* were detected by the CIM (Table 3). The QTL *PsAeAph2-C2.1*, detected in the 2015 field (DSF15) experiment and the pooled GH (DSGHC) data, and a second QTL *Ps.AeMRDC1-C2.2*, detected in the 2016 field (DSF16) experiment, explained 11.2-17.3% and 14.1% of the total variation in ARR severity, respectively. The QTL *Ps.AeMRDC1-C3.1*, detected in GH experiment 2 (DSGH2) and the DSGHC data, explained 23.6% and 12.0% of the phenotypic variance, respectively. *Ps.AeMRDC1-C4.1*, detected in four (DSF16, DSGH1, DSGH2 and DSGH3) of the five experiments (except DSF15), as well as in the pooled field (DSFC) and GH (DSGHC) data), accounted for 16.6%, 13.4%, 13.5%, 16.0%, 16.5% and 36.0% of the phenotypic variance, respectively. *Ps.AeMRDC1-C7.1* and *Ps.AeMRDC1-C7.2*, detected in DSF16 experiment, explained 17.2% and 11.2% of the phenotypic variance, respectively.

The QTL *Ps.AeMRDC1-C2.1* mapped to the top segment of chromosome II (peak marker, 17.9-18.9 cM) flanked by the SNP markers PsCam043049_27080_440 and PsCam000084_71_191. Similarly, *Ps.AeMRDC1-C2.2* also mapped to the top segment of chromosome II (44.6 cM), flanked by the SNP markers PsCam001836_1503_988 and PsCam045443_29114_1358 (Figure 2a). *Ps.AeMRDC1-C3.1* mapped to the middle segment (171-179 cM) of chromosome III, flanked by the SNP markers PsCam048377_31112_1947 and PsCam036430_21570_962 (Figure 2b). *Ps.AeMRDC1-C4.1* mapped to the top-to-middle segment (30.0-47.0 cM) of chromosome IV, flanked by the SNP markers PsCam035653_20827_1413 and PsCam042892_26931_689 (Figure 2c). *Ps.AeMRDC1-C7.1* mapped to the middle segment of chromosome VII (124.3 cM), flanked by the SNP markers PsCam021891_12310_347 and PsCam033643_19217_736, while *Ps.AeMRDC1-C7.2* mapped to the bottom segment of chromosome VII (211.8 cM), flanked by the SNP markers PsCam035721_20895_4786 and PsCam024843_14133_904 (Figure 2d).

The additive effects for *Ps.AeMRDC1-C3.1, Ps.AeMRDC1-C4.1* and *Ps.AeMRDC1-C7.2* had negative values. This indicated that the favourable alleles for partial resistance originated from '00-2067'. In contrast, the additive effects for *Ps.AeMRDC1-C2.1*, *Ps.AeMRDC1-C2.2* and *Ps.AeMRDC1-C7.1* had positive values, indicating that the alleles originated from 'Reward'.

QTL for foliar weight

Ten putative QTL were detected by the CIM for foliar weight in the RIL population inoculated with *A. euteiches* isolate *Ae-MRDC1* (Table 3). *Ps.Fwt-C2.1* detected in the 2016 field (DFF16) experiment and the pooled field (DFFC) data explained 6.5 and 9.9% of the total variation in foliar weight, respectively. *Ps.Fwt-C2.2* detected in only the DFF16 experiment explained 3.5% of the phenotypic variance. *Ps.Fwt-C3.1* was highly consistent across the two field experiments (DFF15 and DFF16), as well as the pooled field (DFFC) and greenhouse (DFGHC) data and explained 1.0%, 2.0%, 4.3% and 2.7% of the phenotypic variance, respectively. The QTL *Ps.Fwt-C4.1*, detected in the DFGH1 experiment and DFGHC data, explained 11.8% and 1.7% of the phenotypic variance, respectively. *Ps.Fwt-C4.2*, detected in DFF16 and DFGH2 experiments as well as the DFGHC data, explained 29.3%, 12.3%, and 18.5% of the phenotypic

variance, respectively. Three QTL, *Ps.Fwt-C6.1*, *Ps.Fwt-C6.3* and *Ps.Fwt-C6.4*, detected in the DFF16 experiment, explained 2.4%, 11.1% and 5.0% of the phenotypic variance, respectively. The QTL *Ps.Fwt-C6.4* also was detected in the DFFC data and accounted for 4.9% of the phenotypic variance. The QTL *Ps.Fwt-C6.2,* detected in DFGH1 experiment and the DFGHC data, each explained approximately 0.3% of the phenotypic variation. *Ps.Fwt-C7.1* detected in the DFGH3 experiment accounted for 15.1% of the phenotypic variance.

The QTL *Ps.Fwt-C2.1* and *Ps.Fwt-C2.2* mapped to the top (38.3-44.5 cM) and middle (91.2 cM) segments of chromosome II, respectively. The SNP markers PsCam051124_33660_880 and PsCam050370_32957_642 flanked *Ps.Fwt-C2.1*, while PsCam011350_7726_1258 and PsCam042179_26280_4473 flanked *Ps.Fwt-C2.2* (Figure 3a).*Ps.Fwt-C3.1* mapped to the middle (143.0-152.0 cM) segment of chromosome III. The SNP markers PsCam038445_23479_407 and PsCam035416_20603_89 flanked *Ps.Fwt-C3.1* (Figure 3b). *Ps.Fwt-C4.1* and *Ps.Fwt-C4.2* mapped to the top (25.0; 39.2-47.9 cM) segment of chromosome IV. The SNP markers PsCam036907_22020_2121 and PsCam037549_22628_1642 flanked *Ps.Fwt-C4.1*, while PsCam054029_357722_104 and PsCam043430_27439_1668 flanked *Ps.Fwt-C4.2* (Figure 3c).*Ps.Fwt-C6.1* and *Ps.Fwt-C6.2* mapped to the top (2.0; 16.7 cM), *Ps.Fwt-C6.3* mapped to the middle (68.4 cM), and *Ps.Fwt-C6.4* mapped to the bottom (145.2 cM) segment of chromosome VI. The SNP markers PsCam001619_1339_512 and PsCam001017_867_763 flanked *Ps.Fwt-C6.1*, PsCam058251_38732_521, PsCam034714_20082_2414 flanked *Ps.Fwt-C6.2*, PsCam011542_7868_781, and PsCam037575_22653_1339 flanked *Ps.Fwt-C6.3,* while PsCam012410_8432_407 and PsCam036548_21683_1851 flanked *Ps.Fwt-C6.4* (Figure 3d). The SNP markers PsCam044214_28123_1477 and PsCam039434_24372_625 flanked *Ps.Fwt-C7.1* (Figure 3e).

The additive effects for *Ps.Fwt-C2.2*, *Ps.Fwt-C3.1*, *Ps.Fwt-C4.1*, *Ps.Fwt-C4.2*, *Ps.Fwt-C6.3* and *Ps.Fwt-C7.1* had positive values. This indicated that the favourable alleles for foliar weight originated from '00-2067'. In contrast, the additive effects for *Ps.Fwt-C2.1*, *Ps.Fwt-C6.1*, *Ps.Fwt-C6.2* and *Ps.Fwt-C6.4* had negative values, indicating that the alleles originated from 'Reward'.

QTL for vigor

Eight putative QTL were detected via CIM for vigor in the RIL population inoculated with *A. euteiches* isolate *Ae-MRDC1* (Table 3). The QTL *Ps.Vig-C1a.1* and *Ps.Vig-C2.1*, detected in the 2015 field (VF15) experiment, explained 12.1% and 11.7% of the phenotypic variance, respectively. *Ps.Vig-C2.2*, detected in the 2016 field (VF16) experiment and pooled field (VFC) data, explained 9.2% and 9.7% of the phenotypic variance, respectively. *Ps.Vig-C3.1* was highly stable, accounting for 7.8% of the phenotypic variance in the VF2016 experiment, 6.8% and 19.3% of the variance in two GH experiments (VGH1 and VGH2, respectively), and 5.2% and 15.7% of the phenotypic variance in the pooled field (VFC) and greenhouse (VGHC) data, respectively. *Ps.Vig-C3.2*, detected in the VF15 experiment, explained 10.2% of the phenotypic variance. *Ps.Vig-C4.1* was highly stable across the VF2016, VGH2, VGH3 and VFC, explaining 28.7%, 19.8%, 26.6% and 22.3% of the phenotypic variance, respectively. *Ps.Vig-C4.2* and *Ps.Vig-C4.3* (located distal to *Ps.Vig-C4.2*), detected in the VGH1 experiment, explained 0.1% and 0.2% of the phenotypic variance, respectively (Table 3).

Ps.Vig-C1a.1, mapped to the top-to-middle segment (49.9 cM) of chromosome I, was flanked by the SNP markers PsCam048937_31589_2232 and PsCam003924_2990_265 (Figure 4a). *Ps.Vig-C2.1* and *Ps.Vig-C2.2* mapped to the top segment (17.9, 44.5 cM) of chromosome II, with the former flanked by the SNP markers PsCam043395_27408_238 and PsCam045802_29408_946 and the latter flanked by the SNP markers PsCam031050_18310_2958 and PsCam013036_8821_1018 (Figure 4b). *Ps.Vig-C3.1* mapped to the middle segment (152.0-191.3 cM) of chromosome III and was flanked by the SNP markers PsCam007018_5220_1401 and PsCam035416_20603_89, while *Ps.Vig-C3.2* mapped to the bottom segment (293.6 cM) of the chromosome and was flanked by the SNP marker PsCam020937_11699_2576 and the SSR marker AA5 (Figure 4c). *Ps.Vig-C4.1* and *Ps.Vig-C4.2* mapped to the top-to-middle segment (51.1-77.2 cM) of chromosome IV, while *Ps.Vig-C4.3* mapped to the bottom segment (163.6 cM) of the chromosome. The SNP markers PsCam001086_922_203 and PsCam027331_15987_254 flanked *Ps.Vig-C4.1, PsCam004871*_3678_1782 and PsCam057281_37909_2940 flanked *Ps.Vig-C4.2*, while PsCam003509_2710_587 and PsCam022603_12702_205 flanked *Ps.Vig-C4.3* (Figure 4d).

The additive effects for *Ps.Vig-C3.1*, *Ps.Vig-C3.2*, *Ps.Vig-C4.1*, *Ps.Vig-C4.2* and *Ps.Vig-C4.3* had positive values, indicating that the favourable alleles for vigor originated from '00-2067'. In contrast, the additive effects for *Ps.Vig-C1a.1*, *Ps.Vig-C2.1* and *Ps.Vig-C2.2* had negative values, which indicated that the alleles originated from 'Reward'.

QTL for plant height

Four putative QTL for plant height were detected by CIM in the RIL population inoculated with *A. euteiches* isolate *Ae-MRDC1* (Table 3). Three of the four QTL, *Ps.Hgt-C3.1*, *Ps.Hgt-C4.1* and *Ps.Hgt-C7.1*, were highly stable across the three GH experiments (HGH1, HGH2 and HGH3) as well as in the pooled greenhouse data (HGHC). The percentage of phenotypic variance explained by *Ps.Hgt-C3.1*, *Ps.Hgt-C4.1* and *Ps.Hgt-C7.1* ranged from 11.6-19.7%, 12.5-17.4% and 16.5-24.9%, respectively. The fourth QTL, *Ps.Hgt-C5a.1* detected in HGH1 experiment explained 6.9% of the phenotypic variance (Table 3).

Ps.Hgt-C3.1 mapped to the bottom segment (287 cM) of chromosome III and was flanked by the SNP marker PsCam020937_11699_2576 and the SSR marker AA5 (Figure 5a). *Ps.Hgt-C4.1* and *Ps.Hgt-C5a.1* mapped to the top segments of chromosome IV (13.7 cM) and VI (1.1 cM), respectively. The SNP markers PsCam000228_198_1085 and PsCam037026_22136_167 flanked *Ps.Hgt-C4.1* (Figure 5b), while the SNP markers PsCam017782_10917_295 and PsCam005127_3886_1505 flanked *Ps.Hgt-C5a.1* (Figure 5c). *Ps.Hgt-C7.1* mapped to the middle segment (124.1-132.3 cM) of chromosome VII, and was flanked by the SNP markers PsCam039102_24080_996 and PsCam060132_40119_811 (Figure 5d).

The additive effects for *Ps.Hgt-C4.1* and *Ps.Hgt-C7.1* had positive values. This indicated that the favourable alleles for height originated from '00-2067'. On the other hand, the additive effects for *Ps.Hgt-C3.1*and *Ps.Hgt-C5a.1* had negative values, indicating that the alleles originated from 'Reward'.

Epistatic interactions for QTL pairs

Three hundred and seventy putative digenic epistatic pairs were detected for root rot severity, dry foliar weight, vigor and plant height in the three greenhouse experiments and the two field experiments conducted in 2015 and 2016 (Data not shown). The number of putative digenic interactions detected in the five environments ranged from 14 to 20, 17 to 27, 17 to 22 and 18 to 24 for root rot severity, dry foliar weight, vigor and plant height, respectively. Of the 370 putative digenic interactions, one of the QTL pairs had a PVE ≥ 15%, 19 had 7.5% ≤ PVE ≤ 15% and 350 had a PVE ≤ 7.5%. In the case of the pooled data, the number of putative digenic interactions detected were 18, 13, 19, 39, 15, 20 and 22 for DSFC, DSGHC, DFFC, DFGHC, VFC, VGHC and HGHC, respectively (Figure 6). Of the 146 digenic interactions detected in the pooled environments, 11 QTL pairs had 7.5% ≤ PVE ≤ 15% and 135 had a PVE ≤ 7.5%. A list of the single significant (PVE ≥ 15%) and 30 moderate (PVE ≥ 7.5%) digenic interactions is presented in Table 4.

Markers for 27 of the 31 major- and moderate-effect epistatic QTL (Table 4) were also linked to 10 additive-effect QTL (*Ps.AeQRCD1-C2.1*, *Ps.AeQRCD1-C4.1*, *Ps.Fwt-C3.1*, *Ps.Fwt-C4.1*, *Ps.Fwt-C4.2*, *Ps.Fwt-C6.3*, *Ps.Vig-C3.1*, *Ps.Vig-C3.2*, *Ps.Vig-C4.1* and *Ps.Hgt-C3.1*) identified in this study. The most significant QTL×QTL interaction, *PsE.AeQRCD1-12*, involved markers linked to three major additive-effect QTL for root rot severity (*Ps.AeQRCD1-C4.1, R^2 =*36.0%), foliar weight (*Ps.Fwt-C4.2*, R^2=29.3%) and vigor (*Ps.Vig-C3.1*, R^2=19.3%), as well as the moderate and minor additive-effect QTL *Ps.Fwt-C4.1* (R^2=11.8%) and *Ps.Fwt-C3.1*(R^2=4.3%), respectively. The second important QTL×QTL interaction, *PsE.AeQRCD1-8*, involved markers linked to three major additive-effect QTL, *Ps.AeQRCD1-C4.1* (R^2 =36.0%), *Ps.Fwt-C4.2* (R^2=29.3%) and *Ps.Vig-C4.1* (R^2=28.7%), as well as the moderate-effect QTL *Ps.Fwt-C4.1* (R^2=11.8%). Two other QTL×QTL interactions, *PsE.Fwt-3* and *PsE.Vig-2*, involved markers linked to two major additive-effect QTL, *Ps.AeQRCD1-C4.1* (R^2 =36.0%) and *Ps.Vig-C4.1* (R^2=28.7%). The QTL×QTL interaction *PsE.Vig-8* involved markers linked to three moderate additive-effect QTL (*Ps.Vig-C3.1*, R^2=19.3%; *Ps.Vig-C3.2*, R^2=10.2%; *Ps.Hgt-C3.1*, R^2=18.7%) and one minor additive-effect (*Ps.Fwt-C3.1*, R^2=4.3%). Markers linked to four QTL×QTL interactions, *PsE.AeQRCD1-2*, *PsE.AeQRCD1-4*, *PsE.Hgt-1* and *PsE.Hgt-2*, were not associated with any of the additive-effect QTL (Table 4).

Discussion

This study evaluated tolerance or partial resistance to ARR in an F_8 RIL population, with this resistance derived from the partially resistant parent '00-2067' (2013). Significant genetic effects within the RIL populations were detected for root rot severity, foliar weight, vigor and plant height. This was probably due to genetic differences in the parents (Conner et al. 2013), which were manifested as diversity alleles in the RIL population. The frequency distribution of the pooled data for all traits in the field trials or greenhouse experiments were continuous, but deviated from normality with various level of skewness and kurtosis, which is not unusual for field disease data (Eskridge 1995; Feng et al. 2013; Coyne et al. 2015). This probably reflected environmental effects and the contribution of many different QTL, each of which was responsible for small increments in the resistance.

Transgressive segregation, in which some lines were more resistant or susceptible than the resistant and susceptible parents, was in the RIL population for both the field and greenhouse experiments. Transgressive segregation has been reported in several studies (Jinks and Pooni 1976; Pilet-Nayel et al. 2002; Feng et al. 2011; Li et al. 2012; Coyne et al. 2015). The factors responsible for transgressive segregation of the progeny remain unclear (Kuczyn'ska et al. 2007). However, Nakedde et al. (2016) suggested resistance genes in the parents residing on different linkage groups could account for the higher levels of tolerance exhibited by some of the RILs. Although the G×E interaction for all traits were significant, moderate to high correlation coefficients were observed for all the traits between field and greenhouse, as well as among individual experiments in the field and greenhouse and in the pooled data. High heritability of traits in each single field or greenhouse experiment as well as in the pooled data also confirmed the significant genetic effects on ARR tolerance in RILs.

We observed high correlation among disease severity and other traits in the field trials and greenhouse experiments (Table S1). Previous studies in chickpea (*Cicer arietinum*) (Johansen et al., 1994) and snap bean (*Phaseolus vulgaris*) (Navarro et al. 2008) found that early vigor had a beneficial effect on shoot biomass production. The epistatic (QTL×QTL) analysis showed a significant interaction of genomic regions linked to root rot severity (*Ps.AeQRCD1-C4.1, R^2 =*36.0%), foliar weight (*Ps.Fwt-C4.2*, R^2=29.3%) and vigor (*Ps.Vig-C3.1*, R^2=19.3%; *Ps.Vig-C4.1*, R^2=28.7%). The observation that about 87% (27 out of 31) of the QTL×QTL interactions were associated with root rot severity, foliar weight and vigor suggests that the same genomic regions control these traits. In the case of height, only 9.7% (3 out of 31) of the QTL×QTL interactions were associated with additive-effect markers for plant height. This suggested that plant height was a poor measure for ARR severity in pea. Thus, the results of our study were consistent with the findings of Conner et al. (2013), which suggested that ARR affected foliar weight and plant vigor in pea but not plant height.

In the QTL mapping, genomic regions corresponding to 28 QTL for root rot severity, two disease-related criteria (foliar weight and vigor) and one agronomic trait (plant height) were identified. The two major-effect QTL (R^2 29.9 to 36.0%) for resistance to *A. euteiches* isolate *Ae-MRDC1* identified in this study were found on the same chromosome as those reported in previous studies. The largest major-effect and most stable QTL *Ps.AeMRDC1-C4.1* (R^2 up to 36.0%) for resistance to ARR detected in this study was located on chromosome IV. The largest major-effect QTL *APh1* (R^2 up to 47%) detected by Pilet-Nayel et al. (2002; 2005) was also located on chromosome IV. Weeden (2000) also reported that a gene influencing tolerance to common root rot in pea was located on chromosome IV. In contrast, the third largest major-effect QTL (*AePs4.1*) detected by Hamon et al. (2011) was located on chromosome IV. The second major-effect QTL for resistance to ARR detected in this study (*PsAe-MRDC1-C3.1*; R^2 up to 23.6%) was located on chromosome III. Similarly, the second major effect QTL detected by Hamon et al. (2011) (*Ae-Ps3.1*; R^2 up to 29.9%) was located on chromosome III. Hamon et al. (2011) detected *Ae-Ps3.1* consistently in multiple experiments, while this study identified *Ps.AeMRDC1-C3.1* in only one of the greenhouse experiments (pooled greenhouse data not counted).

The QTL for resistance to *A. euteiches* isolate *Ae-MRDC1* located on chromosomes II (*Ps.AeMRDC1-C2.1* and *Ps.AeMRDC1-C2.2*) and VII (*Ps.AeMRDC1-C7.1* and *Ps.AeMRDC1-C7.2*) were found to be moderate-effect QTL in this study, with R^2 values ranging from 11.2% to 17.2%. The QTL reported on chromosome II and VII by Hamon et al. (2011) were found to be a combination of minor effect or major effect QTL. *Ae-Ps2.2* was a major-effect QTL (R^2=26.9%), while *Ae-Ps2.1 and Ae-Ps2.3* were minor effect QTL, accounting for up to 15.4% of the phenotypic variation. Similarly, two QTL *Ae-Ps7.6a* and *Ae-Ps7.6b* detected on chromosome VII by Hamon et al. (2011) were moderate effect (R^2 =14.4%) and major-effect QTL (R^2 =42.2%), respectively. Coincidentally, Pilet-Nayel et al. (2002, 2005) also detected two QTL (*Aph6* and *APh13*) on chromosome VII for disease-related criteria, namely above ground index and root weight loss.

Similar to the findings of Pilet-Nayel et al. (2005), most stable and consistently detected QTL with largest R^2 for aboveground disease indices (foliar weight and vigor) were located on chromosome IV, with R^2 values of up to 29.3% and 28.7%, respectively. Therefore, the major-effect and moderate-effect QTL detected in this study and those reported by Weeden et al (2000), Pilet-Nayel et al. (2002; 2005) and Hamon et al. (2011) are very similar.

The similarity in the number of major and moderate QTL detected in this and earlier studies may reflect the fact that commercial pea cultivars have been developed from a very limited pool of partially resistant pea germplasm (Lockwood and Ballard 1960; Shehata et al. 1983; Gritton 1990, 1995; Kraft 1992; Davis et al. 1995; Wicker et al. 2001, 2003; Roux-Duparque et al. 2004; Pilet-Nayel et al. 2007). One of the progenitors of the tolerant parent '00-2067' used in this study was the plant introduction (PI) line 257593. PI 257593 was reported to be highly resistant to root rot caused by Fusarium and Pythium species (Kraft 1974). Only a handful of workers have focused on the development of pea cultivars partially resistant to root rot-causing pathogens including *A. euteiches* (Lockwood 1960; Kraft 1974, 1984, 2001; Kraft and Burke 1974; Kraft and Giles 1976, 1978; Gritton 1990; Davis et al. 1995; Gritton 1995; Kraft and Coffman 2000a, b; Roux-Duparque et al. 2004; Pilet-Nayel et al. 2002, 2005, 2007). Many breeding programs around the world have utilized the few partially resistant progenitor pea germplasm from North America. Some of the partially resistant germplasm might have also been crossed with each other to stack the resistance genes. Hence, it is likely that the partially resistant parent '00-2067' used in this study may share some common genetic basis with pea germplasm used in the previously reported studies.

Marker-assisted selection requires the development of molecular markers either from the gene controlling the trait under study or from genomic regions flanking the gene. However, unlike qualitative traits involving dominant genes, MAS has not always been successful for quantitative traits involving polygenic genes (Xu and Crouch 2008; Hospital 2009). An obvious challenge is the stacking the many genes controlling a complex trait into a single germplasm. In addition, many QTL are unstable in different environments, even if they have large effects, and negative epistatic interactions may reduce the efficiency of MAS (Hospital 2009). In this study, the genomic regions corresponding to *Ps.AeMRDC1-C4.1, Ps.Fwt-C4.2* and *Ps.Vig-C4.1* were within ≈ 0.0-20.0 cM of each other. The co-localization of root rot severity, foliar weight and vigor suggest that this region is very important for the resistance of pea to ARR. Therefore, targeting the 38.0-58.0 cM region on chromosome IV may be an important breeding objective. Based on our linkage map, the SNP markers PsCam037549_22628_1642 and PsCam026054_14999_2864 bordered this region. The region contained 80 additional SNP markers (i.e., 4 markers/ cM). Markers in this region belonged to linkage disequilibrium (LD) block *IV.8* in study of Desgroux et al. (2016). Genes in LD block *IV.8* included an LRR serine threonine protein kinase and vacuolar amino acid transporter, which are involved in the plant defense response, the FYVE zinc finger domain involved in signal transduction, AP2-like ethylene-responsive and bHLH123 transcription factors, and ATPase involved in biochemical and other cellular processes (Desgroux et al. 2016).

In conclusion, linkage analysis and QTL mapping using high-density SNP markers and SSR anchor markers and an F_8 RIL population enabled us to identify a 20.0 cM chromosomal region on chromosome IV as being largely responsible for partial resistance to *A. euteiches* isolate *Ae-MDCR1*. Extensive validation of the identified markers is needed to determine their utility given the challenges associated with MAS of quantitative traits.

Declarations

Funding

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Ethical standards

On behalf of all authors, the corresponding author states that all experiments in this study complied with the ethical standards in Canada.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Consent to participate

Not applicable.

Consent for publication

Not applicable

Availability of data and material

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

Code availability

Not applicable.

Author Contribution Statement

WLF Isolation of *Aphanomyces euteiches* isolate *Ae-MRDC1*, inoculum preparation, greenhouse screening of RIL population and parents for resistance to Aphanomyces root rot, disease rating, measurement of foliar weight, vigor and plant height, phenotypic data analysis, DNA extraction, PCR , genotyping with

SSR markers and writing of the manuscript.

FAR Supervision of molecular marker work, molecular data analysis, linkage map construction, QTL mapping and writing of the manuscript.

HSF Principal investigator, grant application, supervision and provision of technical support to graduate student for RIL population screening in the greenhouse and revision of the manuscript.

CR Development of RIL population, field evaluation of RIL, disease evaluation, measurement of vigor and foliar weight and revision of the manuscript.

CKF Grant application, supervision and provision of technical support to graduate student for RIL population screening in the greenhouse.

MD Grant application and the provision of technical support to graduate student.

SES Principal investigator, grant application, supervision and provision of technical support to graduate student and revision of the manuscript.

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Tables

Table 1. Statistical summary of the traits for the parents (pea cultivars '00-2067' and 'Reward'), the RIL population and the Shapiro-Wilk test based on three greenhouse experiments, field experiments in 2015 and 2016, and the pooled data from the greenhouse and the field experiments.

			'00-2067'	'Reward'		RIL population				
Trait	Expt	Abbrev.	LSM	LSM	P value	LSM	G effect	Skewness	Kurtosis	Shapiro test (*P*)
Root rot severity	GH 1	DSGH1	2.3 ± 1.0	5.0 ± 1.3	2.91E-04	3.7 ± 1.5	4.43E-03	0.2	-0.8	2.39E-02
Root rot severity	GH 2	DSGH2	1.5 ± 1.1	6.1 ± 0.9	3.47E-04	4.7 ± 1.6	8.51E-02	-0.3	-0.5	6.68E-02
Root rot severity	GH 3	DSGH3	1.7 ± 0.6	5.1 ± 0.5	2.33E-04	5.0 ± 1.5	8.53E-03	-0.4	-0.4	1.15E-02
Root rot severity	GH pooled	DSGHC	1.8 ± 0.9	5.3 ± 1.8	8.51E-11	4.5 ± 1.1	2.90E-02	-0.4	-0.3	2.88E-02
Root rot severity	Field 2015	DSF15	6.1 ± 0.4	8.1 ± 0.5	1.04E-07	7.5 ± 0.9	2.38E-07	0.1	-1.2	1.49E-04
Root rot severity	Field 2016	DSF16	5.9 ± 0.6	7.6 ± 1.1	1.39E-03	7.0 ± 1.1	1.35E-05	0	-1.1	1.95E-03
Root rot severity	Field pooled	DSFC	6 ± 0.5	7.9 ± 0.9	1.06E-08	7.2 ± 0.9	2.28E-02	-0.3	-0.2	8.80E-04
Dry foliar weight	GH 1	DFGH1	3.0 ± 0.7	2.3 ± 0.8	1.25E-01	2.7 ± 0.9	0.00E+00	1.6	6.8	1.86E-01
Dry foliar weight	GH 2	DFGH2	2.6 ± 0.5	1.8 ± 0.8	1.34E-01	2.9 ± 1.0	7.20E-02	0.5	0	3.43E-02
Dry foliar weight	GH 3	DFGH3	2.8 ± 0.5	2.4 ± 0.5	1.56E-01	2.1 ± 1.0	3.01E-03	0.7	0.7	1.59E-03
Dry foliar weight	GH pooled	DFGHC	2.8 ± 0.6	2.3 ± 0.9	9.38E-03	2.6 ± 0.9	0.00E+00	0.7	0.7	3.49E-02
Dry foliar weight	Field 2015	DFF15	15.9 ± 11.7	2.7 ± 1.2	3.95E-03	5.4 ± 2.9	0.00E+00	0.9	0.4	4.31E-06
Dry foliar weight	Field 2016	DFF16	5.6 ± 2.1	1.1 ± 0.5	7.80E-06	2.7 ± 2.0	0.00E+00	1.3	1	8.39E-10
Dry foliar weight	Field pooled	DFFC	10.8 ± 9.7	1.9 ± 1.2	5.12E-05	4.1 ± 2.3	0.00E+00	1.1	0.5	1.31E-07
Vigor	GH 1	VGH1	3.2 ± 0.4	2.5 ± 0.5	1.28E-02	2.9 ± 0.7	2.11E-05	-0.2	-1	1.80E-03
Vigor	GH 2	VGH2	3.9 ± 0.2	2.3 ± 0.9	2.03E-03	3.1 ± 0.7	1.13E-06	-0.4	-0.6	3.27E-04
Vigor	GH 3	VGH3	3.9 ± 0.2	2.5 ± 0.5	1.30E-06	3.1 ± 0.6	3.82E-05	-0.4	-0.7	1.05E-03
Vigor	GH pooled	VGHC	3.7 ± 0.4	2.5 ± 0.7	1.58E-09	3.0 ± 0.5	8.93E-03	-0.1	-0.8	5.81E-02
Vigor	Field 2015	VF15	3.7 ± 0.4	1.9 ± 0.3	1.94E-08	2.5 ± 0.9	5.96E-08	0.1	-1.1	7.84E-05
Vigor	Field 2016	VF16	3.7 ± 0.5	1.6 ± 0.7	2.19E-06	2.1 ± 0.9	0.00E+00	0.7	-0.6	5.19E-08
Vigor	Field pooled	VFC	3.7 ± 0.5	1.7 ± 0.6	2.76E-13	2.3 ± 0.8	1.97E-06	0.5	-0.6	4.17E-04
Plant height	GH 1	HGH1	223.6 ± 13.8	179.5 ± 28.7	1.57E-03	172.6 ± 64.0	1.75E-04	0.7	0	6.29E-04
Plant height	GH 2	HGH2	173.0 ± 25.6	140.3 ± 36.4	1.04E-01	212.6 ± 82.9	4.35E-05	0.5	-0.5	7.76E-04
Plant height	GH 3	HGH3	164.2 ± 30.5	157.6 ± 23.1	4.22E-01	184.2 ± 74.9	5.96E-08	0.7	-0.2	4.83E-05
Plant height	GH pooled	HGHC	187.3 ± 35.4	159.3 ± 32.9	7.20E-03	190.4 ± 70.7	4.17E-07	0.6	-0.5	7.95E-05

Table 2. The distribution of single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers on nine linkage groups representing all seven chromosomes of F_8-derived recombinant inbred lines of the cross between the pea lines '00-2067' × 'Reward'

Chromosome	Linkage group	Number of markers used for QTL mapping				Total map length/ (cM)	Marker density/loci
		SNP	SSR	SNP + SSR	Bins		
Ia	1	225	3	228	173	205.5	1.1
Ib	2	59	0	59	32	21.2	2.8
II	3	489	5	494	272	251.6	2.0
III	4	525	7	532	197	395.7	1.3
IV	5	497	2	499	151	211.3	2.4
Va	6	166	0	166	55	69.7	2.4
Vb	7	140	1	141	105	95.8	1.5
VI	8	263	0	263	103	155.6	1.7
VII	9	614	3	617	334	298.5	2.1
Total or Average		2978	21	2999	1422	1704.9	1.8

Table 3. Summary of the QTLs associated with Aphanomyces root rot severity, dry foliar weight, vigor and plant height in 135 F_8-derived recombinant inbred pea lines from the cross between the cultivars 'Reward' × '00-2067' under greenhouse conditions and in field experiments conducted in Morden, MB, in 2015 and 2016.

Identified QTL	Trait	Environment	Chrom	Peak (cM)	Confidence interval (cM)	Left Marker	Right marker	LOD	Additiv
Ps.AeQRCD1-C2.1	Root rot severity	Field 2015	II	18.9	13.8-26.9	PsCam043049_27080_440	PsCam000084_71_191	3.5	0.2962
	Root rot severity	Greenhouse pooled	II	17.9	14.5-26.6	PsCam043049_27080_440	PsCam000084_71_191	5.5	0.3505
Ps.AeQRCD1-C2.2	Root rot severity	Field 2016	II	44.6	43.2-46.8	PsCam001836_1503_988	PsCam045443_29114_1358	4.4	0.4124
Ps.AeQRCD1-C3.1	Root rot severity	Greenhouse Expt 2	III	171.0	159.8-183.8	PsCam048377_31112_1947	PsCam036430_21570_962	5.1	-0.9081
	Root rot severity	Greenhouse pooled	III	179.0	168.9-189.1	PsCam048377_31112_1947	PsCam036430_21570_962	3.9	-0.4034
Ps.AeQRCD1-C4.1	Root rot severity	Field 2016	IV	40.6	38.6-42.5	PsCam037549_22628_1642	PsCam027250_15918_2181	6.3	-0.4948
	Root rot severity	Field pooled	IV	41.2	39.2-42.7	PsCam037549_22628_1642	PsCam027250_15918_2181	5.6	-0.3627
	Root rot severity	Greenhouse Expt 1	IV	30.0	24.3-36.6	PsCam035653_20827_1413	PsCam037549_22628_1642	4.7	-0.6802
	Root rot severity	Greenhouse Expt 2	IV	47.0	45.3-48.3	PsCam034496_19882_640	PsCam000015_11_1425	6.0	-0.6072
	Root rot severity	Greenhouse Expt 3	IV	46.6	43.9-55.2	PsCam027250_15918_2181	PsCam042892_26931_689	4.6	-0.5818
	Root rot severity	Greenhouse pooled	IV	40.6	39.2-41.5	PsCam037549_22628_1642	PsCam006741_5013_603	12.4	-0.6779
Ps.AeQRCD1-C7.1	Root rot severity	Field 2016	VII	124.3	116.8-133.4	PsCam021891_12310_347	PsCam033643_19217_736	5.7	0.5293
Ps.AeQRCD1-C7.2	Root rot severity	Field 2016	VII	211.8	210.8-215.7	PsCam035721_20895_4786	PsCam024843_14133_904	5.2	-0.3791
Ps.Fwt-C2.1	Dry foliar weight	Field 2016	II	44.5	42.9-46.6	PsCam002568_2049_206	PsCam050370_32957_642	5.1	-0.0399
	Dry foliar weight	Field pooled	II	38.31	33.8-47.2	PsCam051124_33660_880	PsCam050370_32957_642	3.9	-0.0666
Ps.Fwt-C2.2	Dry foliar weight	Field 2016	II	91.2	90.3-92.3	PsCam011350_7726_1258	PsCam042179_26280_4473	4.7	0.0124
Ps.Fwt-C3.1	Dry foliar weight	Field 2015	III	150.0	144.9-157.9	PsCam038445_23479_407	PsCam035416_20603_89	4.7	0.0415
	Dry foliar weight	Field 2016	III	150.0	144.7-156.1	PsCam038445_23479_407	PsCam035416_20603_89	8.6	0.0267
	Dry foliar weight	Field pooled	III	152.0	146.4-158.3	PsCam038445_23479_407	PsCam035416_20603_89	7.4	0.0518
	Dry foliar weight	Greenhouse pooled	III	143.0	141.2-146.6	PsCam038445_23479_407	PsCam048377_31112_1947	5.3	0.0154
Ps.Fwt-C4.1	Dry foliar weight	Greenhouse Expt 1	IV	25.0	23.6-33.1	PsCam036907_22020_2121	PsCam037549_22628_1642	7.5	0.3544

	Dry foliar weight	Greenhouse pooled	IV	25.0	23.4-27.8	PsCam036907_22020_2121	PsCam037549_22628_1642	4.5	0.1332
Ps.Fwt-C4.2	Dry foliar weight	Field 2016	IV	47.9	45.5-50.4	PsCam010902_7362_452	PsCam020970_11724_452	11.0	0.1161
	Dry foliar weight	Field pooled	IV	41.2	40.0-51.8	PsCam058405_38855_315	PsCam043430_27439_1668	6.7	0.1021
	Dry foliar weight	Greenhouse Expt 2	IV	39.2	36.5-41.5	PsCam054029_35722_104	PsCam036283_21431_1203	3.4	0.3321
Ps.Fwt-C6.1	Dry foliar weight	Field 2016	VI	2.0	1.2-6.6	PsCam001619_1339_512	PsCam001017_867_763	4.9	-0.0238
Ps.Fwt-C6.2	Dry foliar weight	Greenhouse Expt 1	VI	16.7	16.0-17.1	PsCam058251_38732_521	PsCam034714_20082_2414	4.6	-0.0155
	Dry foliar weight	Greenhouse pooled	VI	16.7	15.9-17.1	PsCam058251_38732_521	PsCam034714_20082_2414	3.8	-0.0071
Ps.Fwt-C6.3	Dry foliar weight	Field 2016	VI	68.4	62.7-72.6	PsCam011542_7868_781	PsCam037575_22653_1339	4.3	0.0871
Ps.Fwt-C6.4	Dry foliar weight	Field 2016	VI	145.2	144.5-146.2	PsCam012410_8432_407	PsCam036548_21683_1851	4.0	-0.0417
	Dry foliar weight	Field pooled	VI	145.2	143.8-146.3	PsCam012410_8432_407	PsCam036548_21683_1851	3.6	-0.0432

Table 3 continued. Summary of the QTLs associated with Aphanomyces root rot severity, dry foliar weight, vigor and plant height in 135 F_8-derived recombinant inbred pea lines from the cross between the cultivars 'Reward' × '00-2067' under greenhouse conditions and in field experiments conducted in Morden, MB, in 2015 and 2016.

Identified QTL	Trait	Environment	Chrom	Peak (cM)	Confidence interval (cM)	Left Marker	Right marker	LOD	Additive
Ps.Fwt-C7.1	Dry foliar weight	Greenhouse Expt 3	VII	141.9	137.3-146.6	PsCam044214_28123_1477	PsCam039434_24372_625	4.7	0.3573
Ps.Vig-C1a.1	Vigor	Field 2015	Ia	49.9	48.5-51.2	PsCam048937_31589_2232	PsCam003924_2990_265	4.7	-0.5847
Ps.Vig-C2.1	Vigor	Field 2015	II	17.9	13.8-23.6	PsCam043395_27408_238	PsCam045802_29408_946	4.0	-0.3065
Ps.Vig-C2.2	Vigor	Field 2016	II	44.5	42.9-45.6	PsCam017034_10588_433	PsCam013036_8821_1018	4.8	-0.2524
	Vigor	Field pooled	II	38.3	34.3-39.2	PsCam031050_18310_2958	PsCam017034_10588_433	4.3	-0.2224
Ps.Vig-C3.1	Vigor	Field 2016	III	153.0	149.3-153.9	PsCam006445_4800_1399	PsCam035416_20603_89	4.5	0.0900
	Vigor	Field pooled	III	152.0	145.2-161.3	PsCam007018_5220_1401	PsCam035416_20603_89	4.2	0.2348
	Vigor	Greenhouse Expt 1	III	184.3	153.3-186.9	PsCam005343_4052_245	PsCam035416_20603_89	11.9	0.2080
	Vigor	Greenhouse Expt 2	III	191.3	150.8-196.7	PsCam005343_4052_245	PsCam035416_20603_89	5.0	0.3521
	Vigor	Greenhouse pooled	III	183.3	169.1-193.5	PsCam005343_4052_245	PsCam035416_20603_89	4.9	0.2034
Ps.Vig-C3.2	Vigor	Field 2015	III	293.6	288.1-297.6	PsCam020937_11699_2576	AA5	3.9	0.4119
Ps.Vig-C4.1	Vigor	Field 2016	IV	54.0	52.5-55.9	PsCam054935_36196_599	PsCam027331_15987_254	10.1	0.5015
	Vigor	Field pooled	IV	51.8	49.8-55.0	PsCam001086_922_203	PsCam042892_26931_689	6.9	0.3772
	Vigor	Greenhouse Expt 2	IV	51.1	48.1-53.2	PsCam001086_922_203	PsCam010470_7041_259	6.8	0.3435
	Vigor	Greenhouse Expt 3	IV	54.3	49.5-56.0	PsCam001086_922_203	PsCam027331_15987_254	8.4	0.2917
Ps.Vig-C4.2	Vigor	Greenhouse Expt 1	IV	77.2	75.8-79.7	PsCam004871_3678_1782	PsCam057281_37909_2940	8.1	0.0572
Ps.Vig-C4.3	Vigor	Greenhouse Expt 1	IV	163.6	152.8-164.3	PsCam003509_2710_587	PsCam022603_12702_205	8.4	0.0239
Ps.Hgt-C3.1	Plant height	Greenhouse Expt 1	III	287.6	286.4-292.5	PsCam020937_11699_2576	AA5	3.5	-27.2974
	Plant height	Greenhouse Expt 2	III	287.6	286.6-292.3	PsCam020937_11699_2576	AA5	5.7	-35.8551
	Plant height	Greenhouse Expt 3	III	288.6	287.4-293.2	PsCam020937_11699_2576	AA5	4.7	-30.4306
	Plant height	Greenhouse pooled	III	287.6	286.4-292.0	PsCam020937_11699_2576	AA5	4.6	-26.8801
Ps.Hgt-C4.1	Plant height	Greenhouse Expt 1	IV	13.7	12.3-14.5	PsCam000228_198_1085	PsCam049105_31749_508	4.7	39.4540
	Plant height	Greenhouse Expt 2	IV	13.7	12.2-14.6	PsCam000228_198_1085	PsCam049105_31749_508	4.2	39.8372
	Plant height	Greenhouse Expt 3	IV	13.7	11.9-16.3	PsCam000228_198_1085	PsCam037026_22136_167	3.8	25.0301
	Plant height	Greenhouse pooled	IV	13.7	12.3-14.5	PsCam000228_198_1085	PsCam049105_31749_508	5.4	38.8187
Ps.Hgt-C5a.1	Plant height	Greenhouse Expt 1	Va	1.1	0.0-3.1	PsCam017782_10917_295	PsCam005127_3886_1505	3.3	-16.1147
Ps.Hgt-C7.1	Plant height	Greenhouse Expt 1	VII	124.1	122.7-124.9	PsCam039102_24080_996	PsCam050039_32647_491	7.2	59.9699
	Plant height	Greenhouse Expt 2	VII	132.3	131.2-134.4	PsCam038582_23600_1599	PsCam060132_40119_811	6.2	39.9182
	Plant height	Greenhouse Expt 3	VII	132.3	131.2-134.2	PsCam038582_23600_1599	PsCam006867_5111_92	8.6	37.2787

	Plant height	Greenhouse pooled	VII	132.3	131.0-134.4	PsCam038582_23600_1599	PsCam033643_19217_736	6.4	34.2948	

Table 4. Summary of the major and moderate digenic epistatic interactions (QTL × QTL) detected for Aphanomyces root rot severity, dry foliar weight, vigor and plant height in three greenhouse experiments and two field experiments conducted in Morden, MB, in 2015 and 2016.

Identified epistaic-effect QTL	Trait	Environment	Chrom	QTL 1 position	Left Marker QTL 1	Right marker QTL 1	Chrom	QTL 2 position	Left
PsE.AeQRCD1-1	Root rot severity	Field 2015	II	10	PsCam043049_27080_440	PsCam000362_321_595	III	185	PsC
PsE.AeQRCD1-2	Root rot severity	Field 2016	III	345	PsCam004460_3351_975	PsCam042783_26826_1395	III	390	PsC
PsE.AeQRCD1-3	Root rot severity	Field 2016	III	390	PsCam029411_17551_1348	PsCam042665_26715_153	VI	60	PsC
PsE.AeQRCD1-4	Root rot severity	Field pooled	III	335	PsCam004460_3351_975	PsCam042783_26826_1395	IV	185	PsC
PsE.AeQRCD1-5	Root rot severity	Greenhouse Expt 1	Ia	35	PsCam000453_395_924	PsCam056543_37344_487	III	170	PsC
PsE.AeQRCD1-6	Root rot severity	Greenhouse Expt 1	III	5	PsCam000647_565_2039	AB64	III	185	PsC
PsE.AeQRCD1-7	Root rot severity	Greenhouse Expt 1	II	245	PsCam001889_1542_1317	PsCam002979_2344_187	III	190	PsC
PsE.AeQRCD1-8	Root rot severity	Greenhouse Expt 2	IV	35	PsCam054029_35722_104	PsCam037549_22628_1642	IV	55	PsC
PsE.AeQRCD1-9	Root rot severity	Greenhouse Expt 2	III	325	AA5	PsCam036163_21311_1095	IV	195	PsC
PsE.AeQRCD1-10	Root rot severity	Greenhouse Expt 3	III	85	PsCam050501_33079_1023	PsCam036791_21914_640	III	165	PsC
PsE.AeQRCD1-11	Root rot severity	Greenhouse Expt 3	III	175	PsCam005343_4052_245	PsCam035416_20603_89	Vb	35	PsC
PsE.AeQRCD1-12	Root rot severity	Greenhouse pooled	III	190	PsCam005343_4052_245	PsCam035416_20603_89	IV	30	PsC
PsE.AeQRCD1-13	Root rot severity	Greenhouse pooled	III	195	PsCam005343_4052_245	PsCam035416_20603_89	Vb	35	PsC
PsE.Fwt-1	Dry foliar weight	Field pooled	III	320	AA5	PsCam036163_21311_1095	III	345	PsC
PsE.Fwt-2	Dry foliar weight	Field pooled	III	185	PsCam005343_4052_245	PsCam035416_20603_89	VII	205	PsC
PsE.Fwt-3	Dry foliar weight	Field pooled	IV	55	PsCam010470_7041_259	PsCam042892_26931_689	VII	205	PsC
PsE.Fwt-4	Dry foliar weight	Greenhouse Expt 2	Ia	130	PsCam026762_15513_1619	PsCam051365_33870_1790	VI	75	PsC

Table 4. Summary of the major and moderate digenic epistatic interactions (QTL × QTL) detected for Aphanomyces root rot severity, dry foliar weight, vigor and plant height in three greenhouse experiments and two field experiments conducted in Morden, MB, in 2015 and 2016.

Identified epistatic-effect QTL	Trait	Environment	Chrom	QTL 1 position	Left Marker QTL 1	Right marker QTL 1	Chrom	QTL 2 position	Left Marker
PsE.Vig-1	Vigor	Field 2016	VI	55	PsCam011542_7868_781	PsCam042529_26584_303	VI	85	PsCam042
PsE.Vig-2	Vigor	Field pooled	II	230	PsCam046005_29564_79	PsCam000407_358_479	IV	55	PsCam010
PsE.Vig-3	Vigor	Greenhouse Expt 1	Ia	25	PsCam001003_854_449	AB28	III	170	PsCam005
PsE.Vig-4	Vigor	Greenhouse Expt 1	Ib	10	PsCam011366_7739_322	PsCam000507_444_666	III	175	PsCam005
PsE.Vig-5	Vigor	Greenhouse Expt 1	III	170	PsCam005343_4052_245	PsCam035416_20603_89	VII	50	PsCam017
PsE.Vig-6	Vigor	Greenhouse Expt 2	III	165	PsCam005343_4052_245	PsCam035416_20603_89	III	375	PsCam019
PsE.Vig-7	Vigor	Greenhouse Expt 2	II	15	PsCam043049_27080_440	PsCam000362_321_595	III	190	PsCam005
PsE.Vig-8	Vigor	Greenhouse Expt 2	III	180	PsCam005343_4052_245	PsCam035416_20603_89	III	255	AB68
PsE.Vig-9	Vigor	Field pooled	Ia	10	PsCam029308_17474_1688	PsCam059970_39995_201	III	170	PsCam005
PsE.Vig-10	Vigor	Field pooled	III	170	PsCam005343_4052_245	PsCam035416_20603_89	IV	40	PsCam048
PsE.Vig-11	Vigor	Field pooled	III	165	PsCam005343_4052_245	PsCam035416_20603_89	Va	25	PsCam025
PsE.Vig-12	Vigor	Field pooled	III	175	PsCam005343_4052_245	PsCam035416_20603_89	VII	295	PsCam036
PsE.Hgt-1	Plant height	Greenhouse Expt 3	III	340	PsCam004460_3351_975	PsCam042783_26826_1395	III	345	PsCam004
PsE.hgt-2	Plant height	Greenhouse Expt 3	III	340	PsCam004460_3351_975	PsCam042783_26826_1395	VII	105	PsCam048

Figures

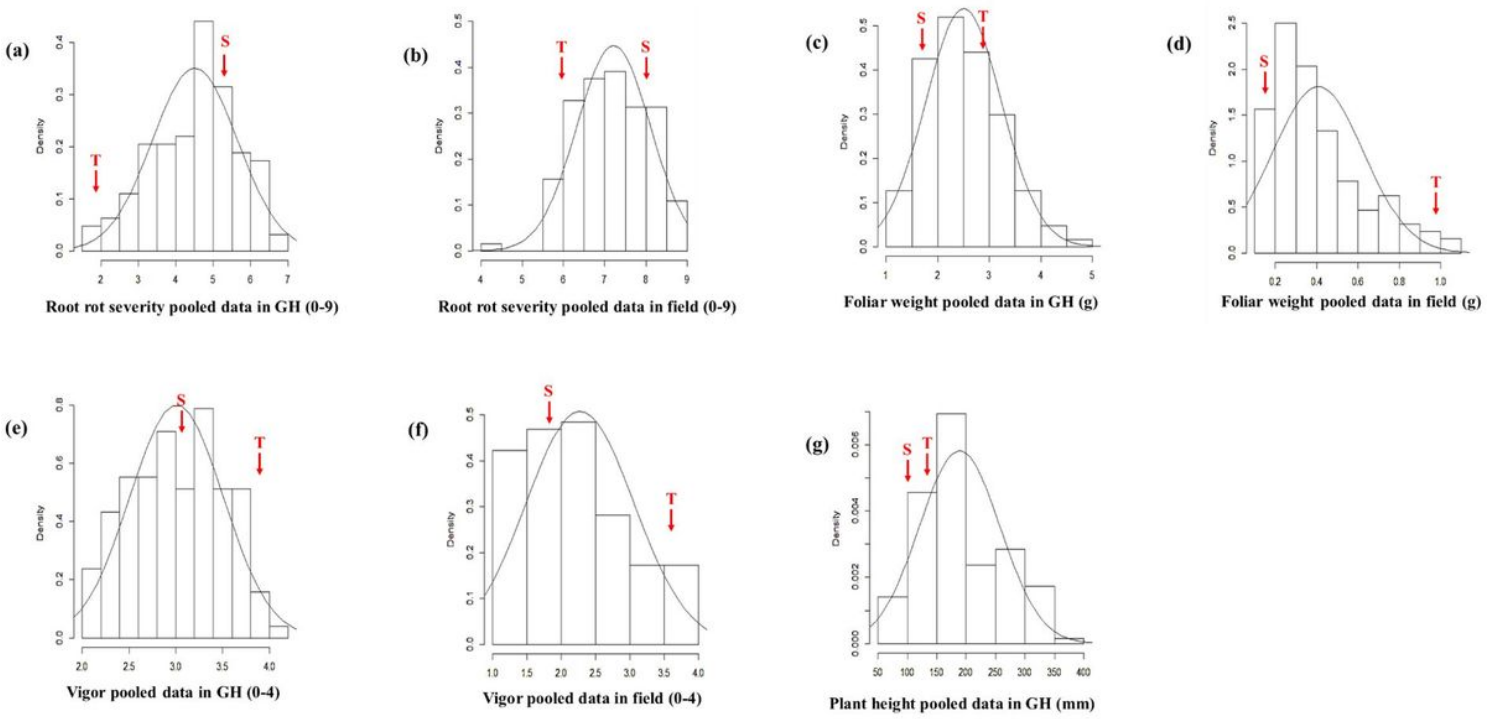

Figure 1

Frequency distribution of pooled data based on least square means for Aphanomyces root rot severity and other traits in field pea. Root rot severity in the (a) greenhouse and (b) field; dry foliar weight in the (c) greenhouse and (d) field; plant vigor in the (e) greenhouse and (f) field; and plant height in the (g) greenhouse. The curves are normal distribution using the mean and standard deviation of the original data. Significant differences between the partially resistant or tolerant parent '00-2067' and susceptible parent 'Reward' are indicated with red arrows.

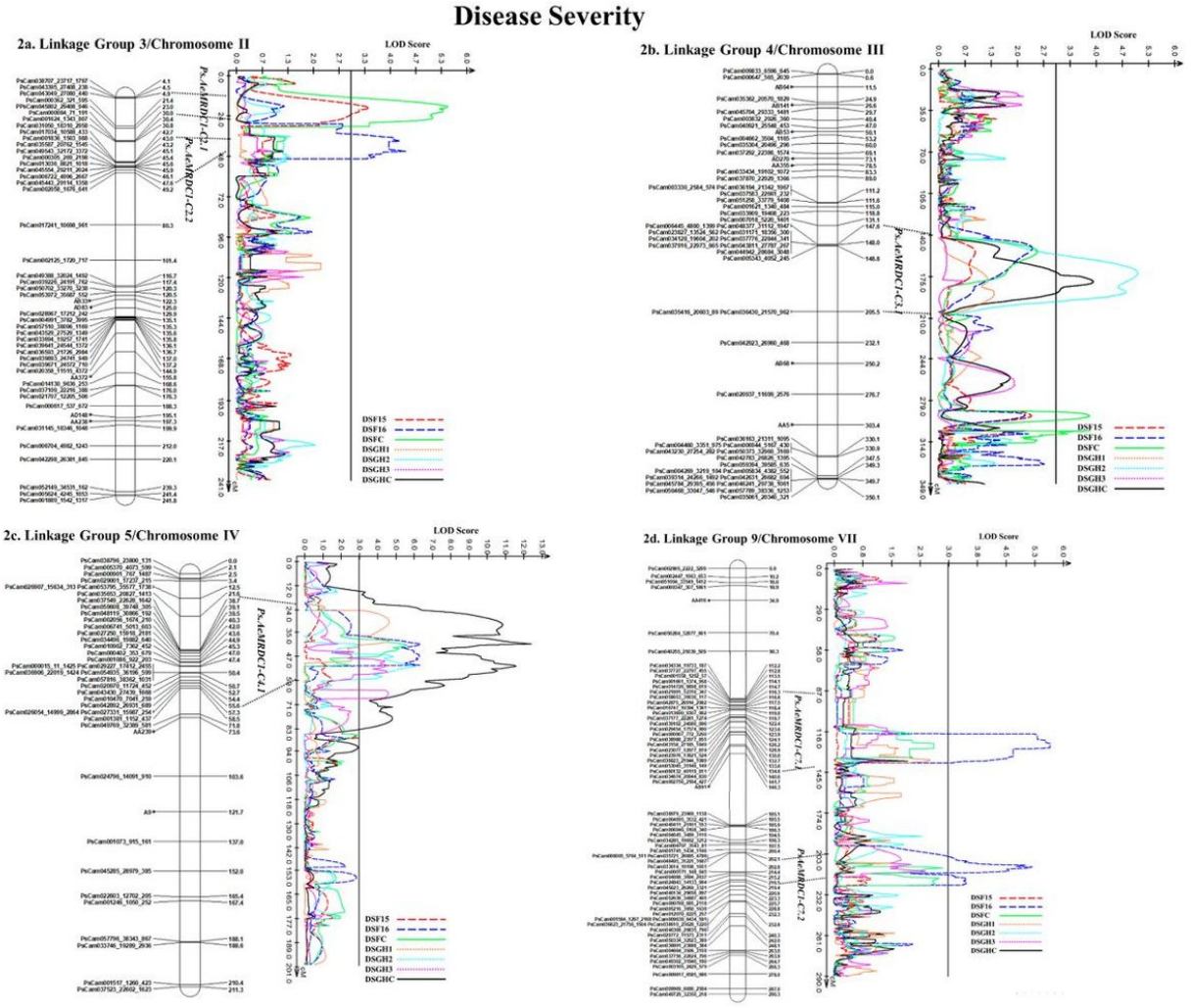

Figure 2

QTL likelihood profile and linkage map of pea chromosomes II, III, IV and VII for partial resistance to Aphanomyces root rot in an F8 RIL of the cross 'Reward' × '002067'. The LOD scores are indicated on the x-axis, while the genetic distances (in cM) are indicated on the y-axis. (a) Two moderate-effect QTL on chromosome II were detected, Ps.AeMRDC1-C2.1 in the 2015 field experiment and the combined greenhouse data, and Ps.AeMRDC1-C2.2 in the 2016 field experiment. (b) One moderate-to-major effect QTL Ps.AeMRDC1-C3.1 on chromosome III was detected, in greenhouse experiment 2 and the combined greenhouse data. (c) The largest major-effect QTL Ps.AeMRDC1-C4.1 on chromosome IV was detected in the 2016 field experiment, greenhouse experiments 1, 2 and 3, as well as the combined field and greenhouse data. (d) Two moderate-effect QTL Ps.AeMRDC1-C7.1 and Ps.AeMRDC1-C7.2 on chromosome VII were detected in only the 2016 field experiment.

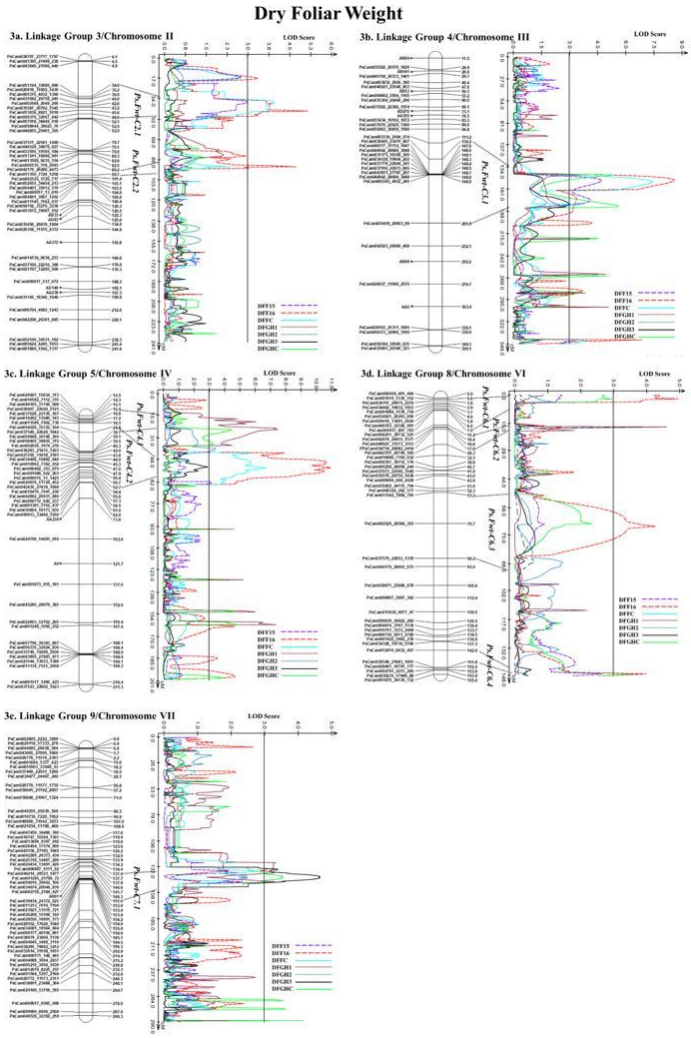

Figure 3

QTL likelihood profile and linkage map of pea chromosomes II, III, IV, VI and VII for foliar weight in an F8 RIL of the cross 'Reward' × '002067'. The LOD scores are indicated on the x-axis while the genetic distances (in cM) are indicated on the y-axis. (a) Two minor-effect QTL on chromosome II were detected, Ps.Fwt-C2.1 in the 2016 field experiment and the combined field data, and Ps.Fwt-C2.2 in the 2016 field experiment. (b) One minor-effect QTL Ps.Fwt-C3.1 on chromosome III was detected in the 2015 and 2016 field experiments and the combined field and greenhouse data. (c) One minor-moderate-effect QTL Ps.Fwt-C4.1 on chromosome IV was detected in greenhouse experiment 1 and in the combined greenhouse data, while the moderate-major-effect QTL Ps.Fwt-C4.2 on the same chromosome was detected in the 2016 field experiment, greenhouse experiment 2 and the combined field data. (d) Four minor-effect QTL were detected on chromosome VI; Ps.Fwt-C6.1 and Ps.Fwt-C6.3 were detected in the 2016 field experiment, Ps.Fwt-C6.2 was detected in greenhouse experiment 1 and in the combined greenhouse data, while Ps.Fwt-C6.4 was detected in the 2016 field experiment and in the combined field data. (e) One major effect QTL Ps.Fwt-C7.1 on chromosome VII was detected in greenhouse experiment 3.

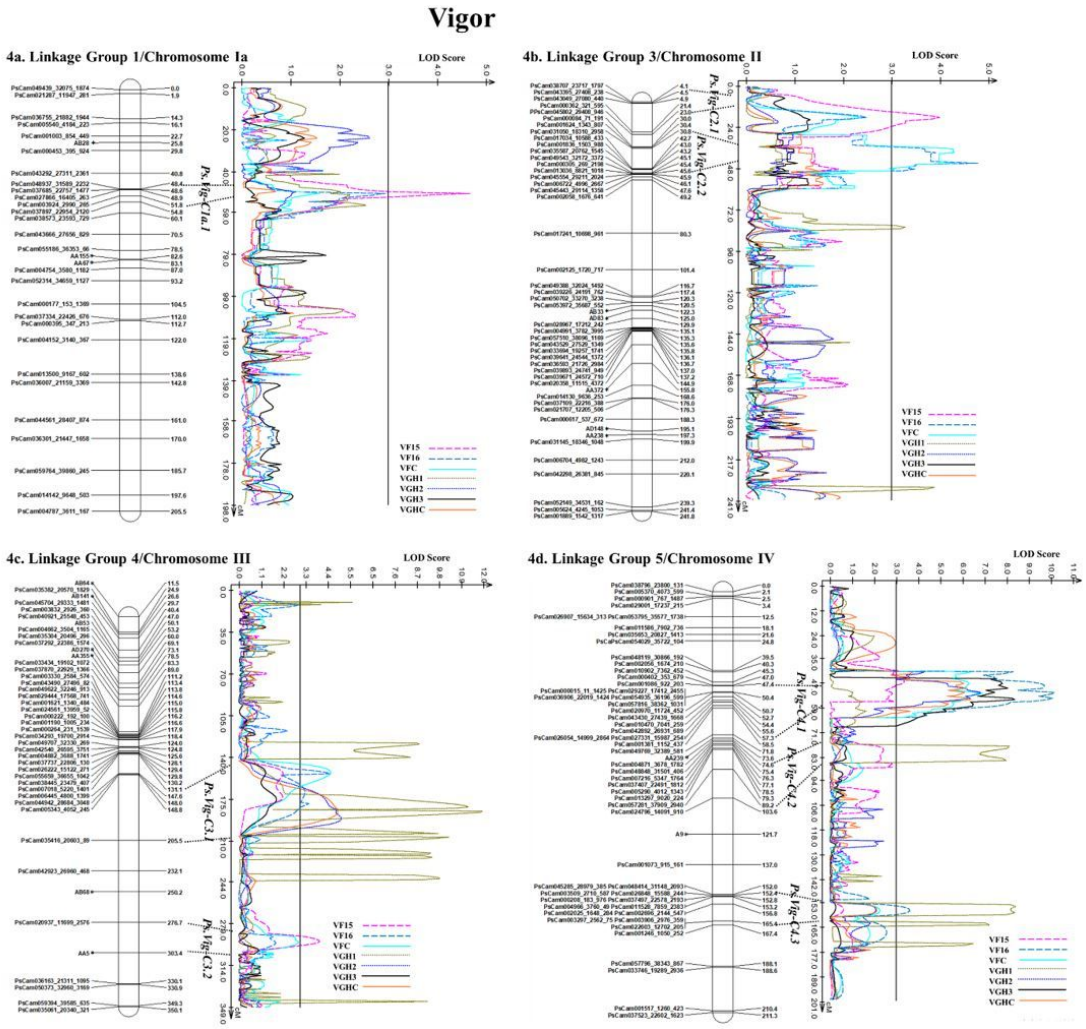

Figure 4

QTL likelihood profile and linkage map of pea chromosomes Ia, II, III and IV for vigor in an F8 RIL of the cross 'Reward' × '002067'. The LOD scores are indicated on the x-axis while the genetic distances (in cM) are indicated on the y-axis. (a) One moderate-effect QTL Ps.Vig-C1a.1 on chromosome Ia was detected in the 2015 field experiment. (b) Two QTL were detected on chromosome II; the moderate-effect QTL Ps.Vig-C2.1 was detected in the 2015 field experiment, while the minor effect QTL Ps.Vig-C2.1 was detected in the 2016 field experiment and the combined field data. (c) Two moderate-effect QTL were detected on chromosome III; Ps.Vig-C3.1 was detected in the 2016 field experiment and the greenhouse experiments 1 and 2, as well as the combined field and greenhouse data, while Ps.Vig-C3.2 was detected in the 2015 field data. (d) The largest major-effect QTL Ps.Vig-C4.1 on chromosome IV was detected in the 2016 field experiment, greenhouse experiments 2 and 3, as well as the combined field data. (d) Two minor-effect QTL Ps.Vig-C4.2 and Ps.Vig-C4.3 on chromosome IV were detected only in greenhouse experiment 1.

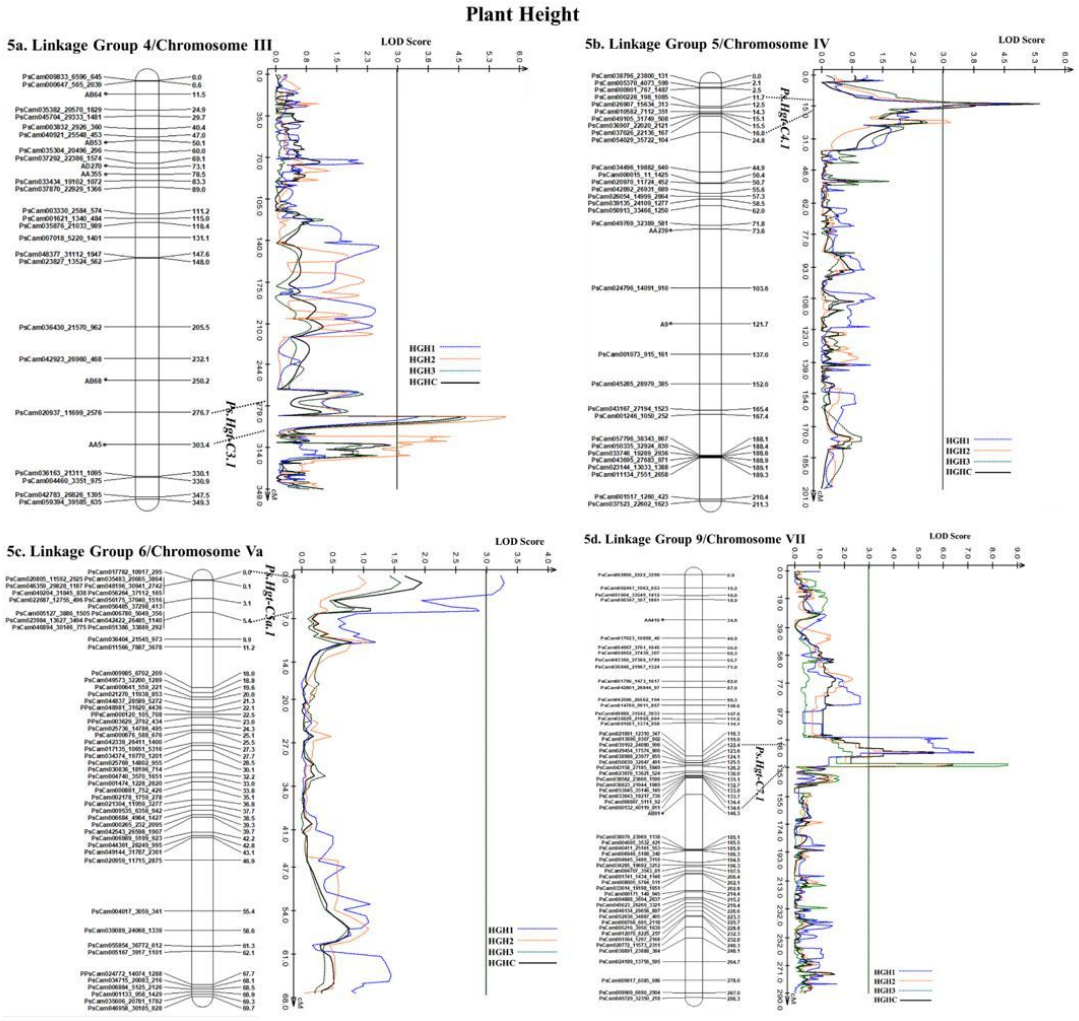

Figure 5

QTL likelihood profile and linkage map of pea chromosomes III, IV, Va and VII for height in an F8 RIL of the cross ‘Reward’ × ‘002067’. The LOD scores are indicated on the x-axis while the genetic distances (in cM) are indicated on the y-axis. The QTL for plant height Ps.Hgt-C3.1 (moderate-effect QTL), Ps.Hgt-C4.1 (moderate-effect QTL), Ps.Hgt-C5a.1 (minor-effect QTL) and Ps.Hgt-C7.1 (major-effect QTL) located on chromosomes III, IV, Va and VII were detected consistently in all three greenhouse experiments and the combined greenhouse data.

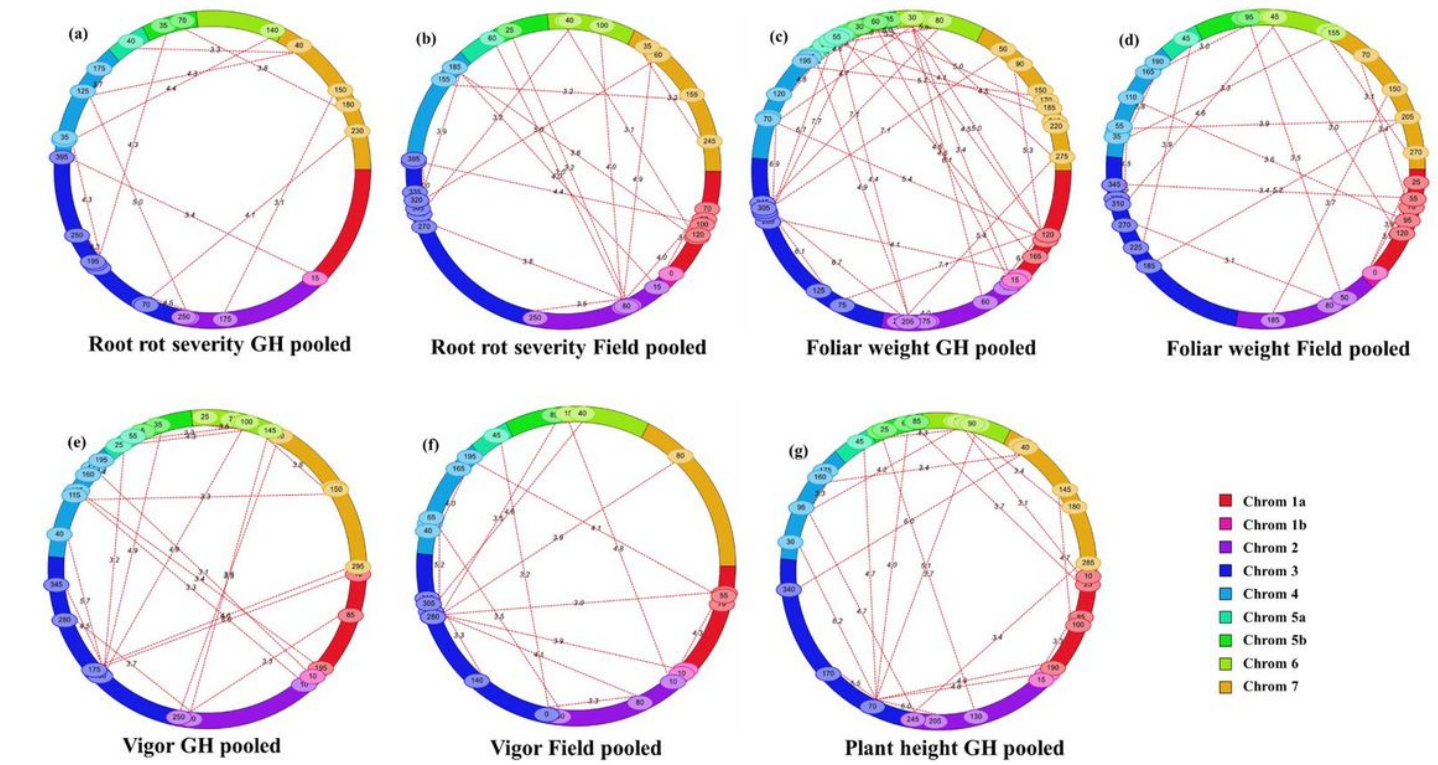

Figure 6

Epistatic QTL conferring tolerance to Aphanomyces root rot of pea in the pooled (a) greenhouse and (b) field data; dry foliar weight in the pooled (c) greenhouse and (d) field data; vigor in the pooled (e) greenhouse and (f) field data; and plant height in (g) the pooled greenhouse data, as indicated by QTL IciMapping V4.1 software. The dashed lines represent epistatic interaction pairs of epistatic QTL, while the numbers represent the LOD scores.

Supplementary Files

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

- TableS1.docx
- TableS2.xlsx