Mapping QTL associated with partial resistance to Aphanomyces and Fusarium root rot in field pea and evaluation of *Aphanomyces euteiches* resistance by bulked segregant RNA-seq

by

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Abstract

Root rot, caused by *Aphanomyces euteiches* and *Fusarium* spp., is an important disease of field pea (*Pisum sativum*) that can be managed with polygenic resistance. An F_8 recombinant inbred line (RIL) population derived from the cross 'Reward' × '00-2067' was evaluated for reaction to *A. euteiches* under field and greenhouse conditions. Genotyping was conducted with a 13.2K single-nucleotide polymorphism (SNP) array and 222 simple sequence repeat markers. Significant genotypic effects and G×E interactions were detected in all experiments. Mapping of quantitative trait loci (QTL) identified 8 major-effect (R^2 >20%), 13 moderate-effect (10%<R^2<20%) and 6 minor-effect (R^2<10%) QTL. A genomic region on chromosome 4 was most consistently responsible for partial resistance to *A. euteiches*.

To evaluate resistance to *Fusarium* spp., the cultivars 'Reward' and '00-02067' were screened with *Fusarium solani*, *F. avenaceum*, *F. acuminatum*, *F. proliferatum* and *F. graminearum* under greenhouse conditions. Significant differences in root rot severity were found between the cultivars in response to *F. avenaceum* and *F. graminearum*, and these species were tested against the F_8 RIL population. While no significant QTL were detected following inoculation with *F. avenaceum*, 5 QTL for root rot severity and 3 QTL each for vigor and plant height were identified for *F. graminearum*. The two most stable QTL for partial resistance to *F. graminearum* were located chromosome 4, coinciding with the region associated with partial resistance to *A. euteiches*. This region may be important for root rot resistance breeding and marker development.

Resistance to *A. euteiches* was evaluated further with another RIL population derived from the cross 'Carman' × '00-2067'. Strongly resistant (R) and susceptible (S) individuals were used to construct R and S bulks, respectively, for bulked segregant RNA-seq (BSR-seq) analysis. Approximately 4.3-5.1 GB read pairs were aligned to the pea reference genome and 44,757 genes examined for expression level, 2,356 of which were differentially expressed. In total, 344.1 K SNPs were detected between the R and S bulks, with 395 variants located in 31 candidate genes. The identification of novel genes associated with partial resistance to *A. euteiches* may facilitate efforts to improve management of this pathogen.

Preface

This thesis is an original work by Mr. Longfei Wu prepared in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Mr. Wu conducted all the experiments and analyzed all of the data in Chapters 2, 3 and 4. The first drafts of all chapters in this dissertation were prepared by Mr. Longfei Wu, and then reviewed and edited by Dr. Stephen E. Strelkov, Dr. Rudolph Fredua-Agyeman, Dr. Sheau-Fang Hwang, and Dr. Kan-Fa Chang, who also developed the original research concept and made suggestions and editorial changes for each chapter. While Mr. Wu was responsible for the data collection and analysis, he received assistance from technical personnel and summer students at the University of Alberta in establishing and maintaining the field and greenhouse experiments.

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Mr. Wu conducted the greenhouse experiment, and collected and analyzed the data. Drs. Hwang, Strelkov and Chang contributed to development of the research concept and provided funding support and guidance. Dr. Fredua-Agyeman assisted with the linkage analysis. All authors contributed to manuscript revision, modification and paper submission.

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List of Abbreviations

Abbreviation	Definition
AAFC	Agriculture and Agri-Food Canada
AFLP	amplified fragment length polymorphism
AM	arbuscular mycorrhizal
ARR	Aphanomyces root rot
BLUPs	best linear unbiased predictors
BSR-seq	bulked segregant RNA sequencing
CIM	composite interval mapping
DEGs	differentially expressed genes
ET	ethylene
ETI	effector-triggered immunity
FHB	Fusarium head blight
FPKM	fragments per kilobase per million
FRR	Fusarium root rot
FSSC	*Fusarium solani* species complex
GO	gene ontology
GWAS	genome wide association study
ISSR	inter-simple sequence repeat
JA	Jasmonic acid
KASP	Kompetitive Allele Specific PCR
LG	linkage group
LOD	logarithm of odd
LSM	least square mean
MAS	marker-assisted selection
MBV	metalaxyl-benomyl-vancomycin
MSTMap	minimum spanning tree map
NGS	next-generation sequencing
NILs	near-isogenic lines
PAMP	pathogen-associated molecular patterns
PCNB	peptone-pentachloronitrobenzene
PDA	potato dextrose agar
PI	plant introduction
PRRC	pea root rot complex
PTI	pattern-triggered immunity
PVE	phenotypic variation explained
QTL	quantitative trait loci
RAPD	random amplified polymorphic DNA
RCBD	randomized complete block design
RILs	recombinant inbred lines

RNA-seq	RNA sequencing
RRS	root rot severity
SA	salicylic acid
SNP	single nucleotide polymorphism
SSD	single-seed descent
SSR	simple sequence repeat
STS	sequence-tagged site

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Chapter 1 General introduction and literature review

1.1 Field pea

Field pea, also called 'dry pea' (*Pisum sativum* var. *arvense* L.), is a globally important pulse crop. Like beans, lentils and chickpeas, field pea is a legume (Bekkering 2013), and like other legumes, it has the ability to fix nitrogen. It is a cool-season, herbaceous, annual crop with a bushy climbing habit, and produces edible seeds in its pods. Field pea is a significant source of food and feed, its seeds being protein-rich and having as much as 86-87% total digestible nutrients (Lazányi 2002). Field pea was first introduced to Canada well over a century ago, and Canada is now the largest producer and exporter of this crop (Slinkard et al. 1994; Wu et al. 2018). In 2020, Canada produced 4.5 million metric tons of field pea, 82% of which was exported to other countries, contributing at least $1.5 billion CAD to the national economy (Agriculture and Agri-Food Canada 2021).

1.1.1 Origin and biology

Pea was domesticated in the Near East around 7000 to 6000 B.C.E., with cultivation in Europe starting around 5500 B.C.E. (Zohary and Hopf 1973). Thomas Knight developed the modern pea in the late 1700s through hybridization with wild peas (Wakelin 2002). It is very difficult, however, to describe pea species, due to the large variation in their morphology and physiology (Hagedorn 1984). Modern pea traits include a smooth and thin seed coat with high germination rates (Zohary and Hopf 1973; Abbo et al. 2011).

Pea development includes four stages: germination, vegetative growth, reproductive growth, and senescence (Knott 1987). Field pea is a dicotyledonous crop with seeds that need 10 to 14 days to germinate. There are two leaf types: normal leafed type forms with several leaflets, a terminal tendril and plant height of 0.9-1.8 meter; and semi-leafless type forms with several tendrils that replace the leaflets, and a shorter height than the normal leafed type. The root system consists of one taproot up to 0.6 m deep and secondary branches that are centered about 0.6 m underground. Nodules, where nitrogen fixation occurs, develop on pea roots after infection and stimulation by

Rhizobium spp. Field pea can adapt to various soil types from slight sandy to heavy clay, but do poorly in highly saline soils and fields with poor drainage. The pea is a self-pollinated plant usually producing white to purple flowers, 40-50 days after seeding; the flowers are heat sensitive. The ambient temperatures during development can influence pod number and seed set. Pods mature within 30 days after fertilization and the seeds are harvested once totally dry.

1.1.2 Importance as a crop

While the production of pea in Canada is overshadowed by the much larger acreages of canola and wheat, field pea is nonetheless an important component of western Canadian cropping systems. The cultivation of pea enhances soil fertility and reduces the need for nitrogen fertilizer, given the ability of this crop to fix nitrogen. Field pea can be grown under both conventional tillage and no-tillage conditions, the latter of which reduces the cost of field preparation for farmers (Bekkering 2013). At present, there is a trend to replace black fallow (bare soil) with green fallow, and pea can be helpful as a cover crop (Schlegel and Havlin 1997). It maintains moisture levels, reduces soil erosion and improves soil quality (Endres et al. 2016). The inclusion of field pea, therefore, is recommended in crop rotations, where it can also reduce disease and pest outbreaks associated with the continuous planting of canola and cereals.

1.1.3 Field pea production

Pea is the third most important legume crop, after bean and lentil, and plays an essential role in agricultural development worldwide. The main pea-producing regions include France, Russia, Ukraine, Denmark and the United Kingdom in Europe; China and India in Asia; Canada and the USA in North America; Chile in South America; Ethiopia in Africa; and Australia (Lazányi 2002; Wu et al. 2019; Wu et al. 2021). Pea production in most regions peaked during the late 1980s and early 1990s with increasing market demand (Lazányi 2002). During this period, Canada, Australia and France were the major exporters of field pea. In the following decades, the situation with respect to field pea cultivation has varied, but Canada continues to play a leading role in production and export. As of 2019, Canada was the largest producer of field pea globally (30% of world

production), followed by Russia (17%), China (10%) and the USA (7%) (FAOSTAT 2019).

The Prairies, including Saskatchewan, Alberta and Manitoba, represent the main region of pea cultivation in Canada. Until the 1980s, most pea hectares were in Alberta, but in the decades since, Saskatchewan has emerged as the main pea-producing province (Bekkering 2011). In 2020, Canadian field pea production was estimated to be 4.6 million tonnes, over half (54.4%) of which were grown in Saskatchewan, followed by Alberta (40.2%) and Manitoba (5.4%) (Wang 2020). More than 100 cultivars of dry pea have been registered in Canada, most of which have yellow or green cotyledons (Bekkering 2013). The focus of pea breeding efforts has been on the improvement of agronomic characters and yield, as well as greater resistance to abiotic and biotic stresses (Bekkering 2013). Given the benefits of pea as a crop, improved germplasm would benefit Canadian growers and the Canadian economy.

1.1.4 Abiotic and biotic limitations

While field pea is well adapted to Canadian cropping systems, the productivity of the crop can be adversely affected by abiotic and biotic factors. The former includes pod shattering, lodging, heat and water stress. Mature and dry pods shatter easily during harvest, leading to potential yield losses. To minimize shattering, harvest is recommended when the plants have been dried but the seeds retain 25-30% moisture (Endres et al. 2021). Other strategies include selecting cultivars with genetic resistance to pod shattering and applying substances to create semi-permeable membranes to reduce pod shattering (Serafin-Andrzejewska et al. 2021). Lodging is another production limitation, resulting in inconvenience and increased expense during harvest, as well as favouring development of foliar diseases (Tar'an et al. 2003). There have been efforts to breed for lodging-resistant pea cultivars. Very high temperatures (>31 °C) during flowering can also be problematic, reducing bud and flower numbers as well as adversely affecting seed formation (Bueckert et al. 2015). According to Klimek-Kopyra et al. (2017), drought stress can affect seed quality, usually leading to smaller seeds. Pea is also sensitive to excessive water (Solaiman et al. 2007); waterlogging will lead to reduced germination (Zaman et al. 2018) and favor several soilborne pathogens (Williamson-Benavides et al. 2021).

Biotic factors affecting field pea cultivation consist mainly of diseases and pests. Common maladies include Ascochyta diseases, root rot and wilt, powdery mildew, bacterial blight and white mold. Ascochyta diseases consist of foliar blights and foot rots and are caused mainly by *Ascochyta pinodella* (teleomorph: *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr.), *Ascochyta pisi* (teleomorph: *Didymella pisi sp. nov.*), and *Phoma koolunga* sp. *nov.* Davidson et al. (Kraft 1998; Davidson et al. 2009; Liu et al. 2013). Root rot and wilt are also caused by a complex of pathogens, and will be discussed in Section 1.2 below. Powdery mildew (*Erisyphe polygoni* (Vaňha) Weltzien) produces white powdery pustules on the leaves, stems and pods, with severe infection resulting in yield loss and delayed maturity. Bacterial blight (*Pseudomonas syringae* pv. *pisi* and/or *Pseudomonas syringae* pv. *syringae*) usually produces water-soaked lesions on the leaves and stipules. White mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, damages the stems, leaves and pods by formation of lesions. In addition to diseases, insect pests can also hamper pea production. Aster leafhopper (*Macrosteles quadrilineatus*), grasshoppers, cutworms, pea aphid (*Acyrthosiphon pisum*) and the pea leaf weevil (*Sitona lineata*) not only cause direct damage to pea leaves, roots, stems and pods, but can also serve as vectors for some pathogens.

1.2 The root rot complex of field pea

Root rot diseases threaten the productivity of many crops, including legumes such as field pea (Williamson-Benavides et al. 2021). The pea root rot complex (PRRC) is associated with a complex of pathogens (Pfender et al. 2001; Williamson-Benavides et al. 2021), including *Aphanomyces euteiches* Drechsler, *Fusarium* spp., *Pythium* spp., *Phytophthora* spp., and *Rhizoctonia solani* J.G. Kühn (Bailey et al. 2003; Chang et al. 2005, 2013). While the PRRC initially affects the roots, the disruption of the root system eventually affects the entire plant (Figure 1.1A). At the seedling stage, the PRRC reduces germination and causes seedling blight. The surviving plants can also develop soft, water-soaked lesions and may show a reddish, brownish or black discolouration (Figure 1.1C). Due to the destruction of the root system, water and nutrient uptake is reduced and the aboveground tissues become yellowed and/or wilted (Figure 1.1B). In severe infections, yield losses can approach 100%. The key factors affecting the development of the PRRC are moisture and

temperature, with outbreaks of the PRRC favored by poor drainage, soil compaction and warm weather.

Cultural and chemical disease management practices, such as crop rotation and seed treatments, are generally insufficient for management of the PRRC. Many of the causal agents are ubiquitous in the soil and/or may produce long-lived survival structures, reducing the effectiveness of crop rotation. Seed treatments may provide some protection, but this may not be enough under heavy disease pressure and/or may not persist beyond the seedling stage. Genetic resistance generally represents the most effective and practical plant disease strategy, and has been deployed in many crops against a variety of diseases in Canada (Anderson et al. 2015; Chang et al. 2018; Brzostowski et al. 2018; Yu et al. 2017; Fredua-Agyeman et al. 2020; Karim et al. 2020; Bokore et al. 2020, 2022; Kosgey et al 2021). Unfortunately, no pea cultivars fully resistant to the PRRC are available, in either Canada or elsewhere (Wu et al. 2018). The fact that the PRRC is associated with numerous pathogens complicates understanding of the genetic control of the interaction(s). As such, it is important to consider the various components of this complex on their own.

1.2.1 Aphanomyces root rot

1.2.1.1 Epidemiology

Aphanomyces root rot (ARR), caused by the oomycete *A. euteiches*, is regarded as the most destructive root disease of field pea (Kraft and Pfleger 2001) and causes yield loss of up to 86% (Pfender and Hagedorn 1983). The disease was first described by Jones and Drechsler (1925) in the USA, and thereafter reported from Europe including France (Labrousse 1933), Russia (Kotova 1969) and Norway (Sundheim 1972). The severity of ARR in Europe has been increasing in recent decades, likely because of the intensive cultivation of field pea (Wicker and Rouxel 2001). While *A. euteiches* was first identified in Canada in the 1970s (Basu 1973), the occurrence of ARR in Saskatchewan (Banniza et al. 2013) and Alberta (Chatterton et al. 2015) was confirmed only recently. In addition to Europe and North America, ARR damage has been reported in Australia

(Shivas 1989), China (Wu et al. 1992), India (Pande and Rao 1998), Japan (Yokosawa et al. 1974), and New Zealand (Manning and Menzies 1984).

Since *A. euteiches* has a broad host range, it can infect common bean, lentil, and alfalfa in addition to field pea (Gossen et al. 2016), and severe losses have been reported in these crops as well (Hall et al. 2005; Zitnick-Anderson et al. 2020; Delwiche 1987). The pathogen favours moist conditions, including water-saturated soils with poor drainage, heavy clays and compacted soils (Hossain et al. 2012; Gossen et al. 2016; Wu et al. 2018). Nonetheless, infection may occur under drier conditions, including soils with moisture levels as low as 30% (Haenseler 1926; Smith and Walker 1941). While *A. euteiches* can cause disease over a fairly wide temperature range (16-28 °C), optimal temperatures for infection are 22-24 °C (Burke and Mitchell 1968; Burke et al. 1969; Papavizas and Ayers 1974).

1.2.1.2 Symptoms and disease cycle

Aphanomyces euteiches can infect pea and cause symptoms at all growth stages. At the seedling stage, the taproot becomes soft and water-soaked with a grayish color. Later, the whole root system may turn brownish or black and become decayed, with the rootlets (and sometimes even the main roots) becoming slimy and easily sloughed off when pulled from the soil. Root nodulation is reduced greatly by *A. euteiches* infection, in turn reducing nitrogen fixation. Symptoms may spread to the stem, weakening the plant and causing lodging. The decay of the roots reduces water and nutrient absorption from the soil and the entire plant may become yellowed and wilted. Seeds will be smaller or not be produced at all, resulting in reduced quality and yield of the crop.

The pathogen survives in the soil as thick-walled (sexual) oospores. These germinate early in the growing season, producing mycelia for direct root infection or forming sporangia for the production of flagellated zoospores. The formation of sporangia and zoospore production is favored at temperatures of 16-20 °C, and oospore germination in general is enhanced by good soil moisture and the presence of host root exudates (Sekizaki et al. 1993). The germinated zoospores can complete the infection process within several hours, including attachment to the host, encysting

and penetration of the root cortex (Papavizas and Ayers 1974). Once inside the roots, hyphae of the pathogen grow inter or intracellulary, eventually producing haploid antheridia (male gametangia) and oogonia (female gametangia) for sexual reproduction (Papavizas and Ayers 1974). The diploid oospores, formed by the fertilization of the oogonia by antheridia, persist in plant debris after the host plant dies or is harvested, and are eventually released back into the soil as the host tissues decompose. Oospores can survive in the soil for more than 10 years, serving as inoculum for future infections (Pfender and Hagedorn 1983).

1.2.2 Fusarium root rot and wilt

1.2.2.1 Epidemiology

In contrast to *A. euteiches*, *Fusarium* spp. are true fungi. While they are also a major component of the PRRC, on their own they cause Fusarium root rot and wilt. Fusarium root rot was first reported in 1918 in the Midwest of the USA, followed by its identification in Europe in 1923 (Kraft and Pfleger 2001). The disease is now known to occur throughout all the main pea-producing regions worldwide. Surveys in western Canada have indicated the prevalence of *Fusarium* spp. in the PRRC (Chang et al. 2004, 2005; Wu et al. 2018). Many *Fusarium* spp. have been reported to be involved in the 'Fusarium root rot complex' worldwide, including *F. avenaceum*, *F. oxysporum*, *F. culmorum*, *F. redolens*, *F. graminearum*, *F. equiseti*, *F. sporotrichioides* and *F. poae* (Persson et al. 1997; Chang et al. 2004; Gregoire and Bradley 2005; Fernandez 2007; Mathew et al. 2012; Tonnberg 2012; Chen et al. 2014; McLaren et al. 2016; Chittem et al. 2015; Zitnick-Anderson et al. 2018). Yield losses of 30-60% have been reported from *Fusarium* infection of field pea in North America (Basu et al. 1976; Chang et al. 2004).

Fusarium solani f. sp. *pisi* was long considered to be the primary cause of Fusarium root rot (Kraft et al. 1988; King et al. 1960). However, more recent studies suggested that *F. solani* f. sp. *pisi* is actually comprised of several species, referred to as the *Fusarium solani* species complex (FSSC) (O'Donnell 2000). Some isolates of the FSSC were reported to be pea-specific in their virulence, while others were avirulent on field pea (VanEtten 1978; Chitrampalam and Nelson Jr

2015). The FSSC was classified into 10 *formae speciales* based on their pathogenicity on a host differential set (Matuo and Snyder 1973). Chitrampalam and Nelson Jr (2015) found that all isolates of *F. solani* f. sp. *pisi* collected from field pea in North Dakota, USA, were classified into clade III. In Canada, *F. avenaceum* was reported as the most prevalent species in the PRRC, representing up to 80% of all *Fusarium* isolates collected from field pea (Feng et al. 2010). This species has also been reported from North Dakota and Montana, USA, as well as southern Scandinavia (Persson et al. 1997; Chittem et al. 2010). *Fusarium oxysporum* Schl. f. sp. *pisi* Snyd. and Hans has also been reported as a major cause of Fusarium wilt in field pea (Kerr 1962), with four races commonly identified (Haglund and Kraft 2001).

The development and spread of Fusarium root rot is associated with temperature, moisture and soil type. While the ideal temperature for *Fusarium* spp. was reported to range from 25-30 °C (Tu 1994; Hwang et al. 2000), this can differ somewhat among species and isolates. Moderate to high soil moisture favors Fusarium root rot, while soil compaction also contributes to disease development, possibly by increasing host plant stress (Gossen et al. 2016). Conventional tillage, wherein the soil is cultivated more regularly, can accelerate the spread of Fusarium root rot and other soilborne pathogens (Gossen et al. 2016).

1.2.2.2 Symptoms and disease cycle

Fusarium spp. initially infect the taproot as the pea seeds germinate, resulting in reddish brown streaks in the main root and rootlets. The stem can also be attacked, with the development of brick red, dark reddish brown, or chocolate-colored, wedge-shaped lesions. The root lesions caused by *Fusarium* spp. in pea increase susceptibility of the host to other soilborne pathogens involved in the PRRC. As a consequence, *Fusarium* spp. are usually recovered together with *Pythium* spp., *R. solani* and *A. euteiches* (Chatterton et al. 2015; Chang et al. 2016; Wu et al. 2017). The dark red color of the root cortex up to the stem is another symptom of Fusarium root rot, but is not visible unless the root system is pulled from the soil. As the disease progresses, infected plants may become stunted, with wilting and yellowed leaves, ultimately leading to incomplete pod filling and premature senescence.

Fusarium spp. form several types of asexual spores, including microconidia (single or two-celled, smaller, elliptical), macroconidia (multiple cellular, larger, slightly curved) and chlamydospores (thick-walled, pigmented, rounded), the latter of which can persist in plant debris or the soil for up to 5 years. The chlamydospores germinate to produce hyphae which penetrate the epicotyl and hypocotyl. Once inside the host, the fungus spreads through the vascular system, causing a reddish-brown discoloration. Large numbers of spores are produced which serve as inoculum to infect other plants.

1.2.3 Root rot management strategies

The PRRC is a significant challenge to Canadian pea production, given the multiple pathogen species involved, their broad host range, the persistent survival structures produced, and the lack of highly resistant field pea cultivars. There are no completely satisfactory management strategies for the PRRC, although there have been some recommendations. In Saskatchewan, conventional tillage was more successful at suppressing root rot than zero tillage (Bailey et al. 2000). Tillage involving a fall chisel plow and spring flat seedbed preparation, or a fall plow and spring raised seedbed preparation, were reported to result in significantly lower root rot severity than a conventional fall plow and spring flat seedbed preparation (Tu 1986). Rotations may also have some benefit in reducing the severity of the PRRC. However, given the long-lived nature of the survival structures produced by *A. euteiches* and *Fusarium* spp., long rotations are required to have a significant effect. For example, a rotation of 6-8 years in length, which excludes possible alternative hosts such as lentil, chickpea and alfalfa, was suggested for the management of root rot caused by *A. euteiches* (Hossain et al. 2015). Such long rotations may not be acceptable to many farmers in western Canada, who often rely on cash crops such as canola for the biggest economic returns. The planting of cover crops has also been suggested as a cultural strategy for management for the PRRC, with the resulting residues and shifts in soil microbial communities reducing root rot severity (Sarwar et al. 1998). For example, the inclusion of a cruciferous cover crop was reported to significantly reduce root Aphanomyces root rot when combined with high N fertilizer rates (Hossain et al. 2015).

Fungicidal seed treatments may also be used to manage the PRRC. Several fungicides, including fosetyl-Al, fludioxonil, metalaxyl, furakxyl and benalaxyl, were reported to increase the seedling emergence rate and reduce root rot severity caused by *A. euteiches* and *Pythium* spp. under experimental conditions (Chang et al. 2013; Tu 1987; McKay et al. 2003; Oyarzun et al. 1990; Xue 2003). However, Apron (metalaxyl) showed insufficient efficacy for the control of root rot caused by *F. avenaceum*. The only seed treatment registered commercially for the management of *A. euteiches* in Canada is Intego Solo (Valent Canada, Inc. Guelph, Ontario), containing ethaboxam, which received emergency approval in Saskatchewan in 2014. In addition to seed treatments, in-furrow application of prothioconazole, fluopyram and penthiopyrad was found to significantly reduce the severity of root rot caused by *Fusarium* spp. in an American study (Modderman et al. 2018). Various biological control agents, which are often regarded as more 'natural' and ecologically friendly, have also been evaluated for disease management in field pea. The biological control agents *Clonostachys rosea*, *B. pumilus*, *B. subtilis*, *B. cereus*, *B. mycoides*, *P. polymyxa*, *P. cepacia* and *P. fluorescens* have been reported to limit pathogen growth *in vitro* and reduce root rot severity caused by *A. euteiches* under controlled conditions (Xi et al. 1996; Wakelin et al. 2002; Xue 2003). At present, however, no, biocontrol agents are registered for use in the management of the PRRC in Canada.

1.2.4 Disease risk assessment

Once symptoms of the PRRC appear in a crop, it is too late to implement mitigation measures for that year. As such, disease risk assessment is important for the effective management of pea root rot. Factors such as soil type, field history, and disease incidence in an area may all provide indirect measures of the likelihood of disease development. In addition, the identification and quantification of the pathogens involved in the PRRC can provide direct estimates of risk. Traditionally, pathogens were identified by culturing on a growth medium and examination for morphological characters. *Aphanomyces euteiches* is characterized by profusely branched, white coenocytic mycelium, its asexual and sexual reproductive structures (sporangia and oogonia, respectively), and the formation of double-walled oospores on root tissue and growth media

(cornmeal or oatmeal agar). *Fusarium* spp. can be identified, briefly, based on the color of their aerial mycelium, and the presence/absence and size of the spores (macroconidia, microconidia and chlamydospore). Unfortunately, diseased root tissue often contains multiple pathogens with various growth rates, making the identification of slow growing pathogens such as *A. euteiches* difficult. Selective media can help to improve isolation of certain target pathogens; examples include metalaxyl-benomyl-vancomycin (MBV) medium for *A. euteiches* (Pfender et al. 1984) and malachite green agar 2.5 ppm (MGA 2.5) for *Fusarium* spp. (Thompson et al. 2012). Even so, the morphological identification of *A. euteiches* and *Fusarium* spp. can sometimes be challenging, requiring time and expertise to obtain pure cultures and positively make a diagnosis.

In recent years, molecular technologies have been applied to the identification of the pathogens involved in the PRRC. These typically involve DNA extraction and PCR amplification with pathogen-specific primers (Sauvage et al. 2007; Zhao et al. 2016; Zitnick-Anderson et al. 2018). Quantitative PCR analysis with a sequence-specific probe is the most common method to measure the amount of a soilborne pathogen. The most commonly used species-specific probe (161T) and primer set (136F/211R) for the detection and quantification of *A. euteiches* in pea plant and soil samples (Sauvage et al. 2007; Wu et al. 2018) were originally developed by Vandemark et al. (2002), and were based on the ITS1 and ITS4 sequences of the pathogen (White et al. 1990). Several commercial labs offer detection of this pathogen as a commercial service. For the detection of *Fusarium* spp., the ITS region and CPN60 gene sequence have often been targeted (Feng et al. 2010), as has the partial translation elongation factor 1-alpha (TEF1-α) gene (Punja et al. 2017; Chittem et al. 2015; Zitnick-Anderson et al. 2018). Feng et al. (2010) reported that isolates of *F. avenaceum* differing in virulence could be distinguished based on differences in their ITS and CPN60 sequences. Similarly, Zitnick-Anderson et al. (2018) developed multiplex PCR assays to identify and quantify common *Fusarium* spp. recovered from field pea in North Dakota. Multiplex qPCR assays have also been used to determine the co-occurrence of *A. euteiches* and *Fusarium* spp. in this crop, providing a more complete assessment of the prevalence of pathogens associated with the PRRC (Willsey et al. 2018).

1.3 Genetic resistance and the management of root rot of field pea

Considering the limitations of chemical and cultural practices for the management of the PRRC, the development of genetically resistant pea genotypes is highly desirable. While commercial pea cultivars with complete resistance are not available at present, efforts to identify and characterize resistance are underway (Conner et al. 2013, 2014; Bodah et al. 2016; Wu et al. 2018; Williamson-Benavides and Dhingra 2021). While full resistance controlled by major genes may be ideal, disease tolerance or partial resistance controlled by quantitative trait loci (QTL) also may offer an important tool for sustainable management of the PRRC.

1.3.1 Resistance screening

Tolerance to root rot, which was heritable and identified as a quantitative trait, was first reported in some pea lines with unfavorable agronomic characters (Marx et al. 1972; Shehata et al. 1983). The transmission of moderate resistance to *A. euteiches* was also confirmed in later studies, suggesting the potential for pyramiding multiple genes to control ARR (Davis et al. 1995). Kraft and Boge (1996) also observed slow lesion development caused by *A. euteiches* in some pea lines, as well as the suppression of zoospore germination and oospore production. McPhee (2005) screened 330 pea accessions and identified one that was highly resistant. Similarly, Conner et al. (2013) reported a highly tolerant genotype, '00-2067', which was identified in a nursery infested with *A. euteiches*. Resistance to *Fusarium* spp. also appears to be partial and a quantitative trait (Bodah et al. 2016). In a field trial conducted with *F. solani* f. sp. *pisi* in 1992 and 1993, no resistant pea genotypes were identified (Hwang et al. 1995). Grünwald et al. (2003) screened 387 pea accessions from the *Pisum* core collection for resistance to Fusarium root rot, and confirmed only moderate resistance. More recently, several pea genotypes with high levels of partial resistance to *F. solani* f. sp. *pis*, including PI 125673, 5003, 'Banner', 'Carneval', PS05300234, and 'Whistler', have been reported (Bodah et al. 2016). However, the expression of resistance was found to be unstable due to environmental influence, and resistance usually occurred in genotypes with unfavorable agronomic traits (Bodah et al. 2016).

1.3.2 Identification of quantitative trait loci (QTL)

Linkage analysis usually relies on the development of a population derived from the cross of resistant and susceptible parents. Breeders grow 6-10 generations (F6-10) to increase heterozygosity. There are two main types of populations used to identify QTL: (1) recombinant inbred lines (RILs) based on the single-seed descent method by self-pollination; and (2) near-isogenic lines (NILs) based on the recurrent method by backcrossing. Phenotyping assessments most often use root rot severity (RRS) as the main resistance criterion, along with other parameters such as aboveground index (Pilet-Nayel et al. 2002), aerial decline index (Hamon et al. 2011), plant height, and root and foliar weights (Desgroux et al. 2016, 2018). Inoculum sources vary by study, and can include growing plants in a naturally infested nursery, applying a spore suspension to the roots and/or growth medium, or use of infested grain inoculum. Disease nurseries most closely approximate 'natural' conditions, but the results can be affected by environmental factors due to the quantitative nature of the resistance. The application of grain inoculum has been used in both field and greenhouse experiments to evaluate and detect genetic resistance (Hwang et al. 1995; Feng et al. 2010), while spore suspensions are generally used under controlled conditions (Pilet-Nayel et al. 2005; Hamon et al. 2011; Mc Phee et al. 2012; Lavaud et al. 2015; Coyne et al. 2015).

Correlation analyses are carried out to determine the phenotypic criteria that are highly correlated with root rot severity for subsequent QTL determination. The phenotypic data are subjected to analysis of variance (ANOVA) under each environmental condition tested to determine the RIL effect and account for the interaction between genetic effect and environment (G×E). Numerous studies have been conducted with pea and root rot for the identification of QTL (Pilet-Nayel et al. 2002, 2005; Hamon et al. 2011, 2013). For example, Pilet-Nayel et al. (2002) estimated that heritability of root rot resistance ranged from 0.40 to 0.71 in a single environment and from 0.30 to 0.51 across environments. Wille et al. (2020) reported that root rot index and foliar weight had moderate levels of heritability, which varied under different conditions.

Marker-assisted selection (MAS) can accelerate the selection process in breeding programs. Various PCR-based markers have been used for detecting genetic resistance to AAR and FRRC in

pea. These include 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; Mc Phee et al. 2012; Lavaud et al. 2015; Coyne et al. 2015) (Table 1.1). Multiple QTL with minor to major effects have been reported to be associated with partial resistance against *A. euteiches* on all seven pea linkage groups, with the percentage of variance explained as high as 42% and 47% on LGVII and LGIV, respectively (Pilet-Nayel et al. 2002, 2005; Hamon et al. 2011, 2013; Lavaud et al. 2015) (Table 1.1). For resistance to *Fusarium* spp., major-effect QTL against *F. solani* and *F. oxysporum* were detected on LGII and LGIV, respectively (Mc Phee et al. 2012; Coyne et al. 2015) (Table 1.1). However, these QTL were still difficult to apply for MAS because of low marker density and large QTL intervals. With the advent of next-generation sequencing technology, very large numbers of single nucleotide polymorphism (SNP) markers located in gene-encoding regions could be readily identified in pea. For instance, Desgroux et al. (2016) found 52 small-size-interval QTL related to ARR resistance in a 13.2K SNP array in a genome-wide association study, and evaluated the potential roles of candidate genes in conferring resistance to *A. euteiches*. In a subsequent study, Desgroux et al. (2018) enlarged the SNP array, validating the QTL detected in the previous study and identifying novel genomic regions associated with ARR resistance. They also were able to select a highly significant SNP marker in the QTL *Ae-Ps7.6*. Similarly, Coyne et al. (2010) used a SNP array to narrow down the confidence interval of a previously reported major QTL (*Fsp-Ps 2.1*) to 1.2 cM. At present, genetic studies are usually focused on single pathogens involved in the PRRC. While some commonalities have been found in studies of resistance to *A. euteiches* vs. *Fusarium* spp., QTL results are rarely comparable, particularly when using different marker sets.

1.3.3 Sequencing technologies

The first plant to have its genome sequenced was *Arabidopsis thaliana* (L.) (Arabidopsis Genome Initiative 2000), which cost $100 million US and took over 10 years (Goff et al. 2014). However, with the development of next-generation sequencing (NGS) technologies and declines

in sequencing costs, many plant genomes have been obtained in recent years, ranging in size from 400 Mbp to 17 Gbp. These include rice (*Oryza sativa* L.; International Rice Genome Sequencing Project 2005), wheat (*Triticum aestivum* L.; International Wheat Genome Sequencing Consortium 2018), maize (*Zea mays* L.; Schnable et al. 2018), as well as the legumes barrel clover (*Medicago truncatula* Genome Project 2015), *Lotus japonicus* (Sato et al. 2008), chickpea (Varshney et al. 2013), soybean (Schmutz et al. 2012) and pigeonpea (*Cajanus cajan* [L.] Millsp.; Varshney et al. 2011). However, sequencing of the pea genome was challenging due to its large size (around 4.5 Gbp) and highly repetitive sequence (75–97%) (Macas et al. 2007; Smýkal et al. 2012). The first and to date only draft pea reference genome became available in 2019 (Kreplak et al. 2019), although this contains important gaps (Afonin et al. 2020).

DNA sequencing began with Sanger sequencing (chain termination method) and Maxam-Gilbert sequencing (chemical degradation method), but cost-efficient NGS technologies have now been developed on many platforms including Roche/454, Illumina/Solexa, ABI/SOLiD, Ion Torrent, PacBio and Oxford Nanopore (Sahu et al. 2020). Several studies for the identification of genetic markers and development of pea consensus maps have been conducted in recent years using next-generation DNA sequencing technologies (Franssen et al. 2011; Kaur et al. 2012; Duarte et al. 2014). In contrast, RNA sequencing (RNA-seq) based on NGS mainly focuses mainly on mRNA, characterizing the transcriptome to detect candidate genes, measure gene expression and identify genetic variants (Garg and Jain 2013; Zhao et al. 2014). An advantage of RNA-seq analysis is that it can be conducted in the presence or absence of a reference genome (Brautigam et al. 2011), referring to resequencing and *de novo* assembly, respectively. *De novo* assembly can detect vast numbers of expressed genes at particular time-points using NGS reads (Alves-Carvalho et al. 2015). Alves-Carvalho et al. (2015) constructed 20 cDNA libraries from pea samples under different nitrogen conditions and generated a 100 Gb *de novo* assembly to produce a uni-gene set for pea root nodulation. Liu et al. (2015) used *de novo* assembly to characterize the transcriptome of pea seed development. Sudheesh et al. (2015) used RNA-seq for *de novo* assembly of 23 cDNA

libraries, annotating tissue-specific transcriptomes for genetic marker development. Afonin et al. (2020) evaluated symbioses with arbuscular mycorrhizae via *de novo* assembly.

Most pea transcriptomic studies have evaluated agronomic traits, with very limited research on disease resistance genes. Hosseini et al. (2015) used an *M. truncatula* microarray to evaluate the immune response of pea against *A. euteiches* and *Phytophthora pisi*, finding the involvement of common and specific signaling pathways. This approach worked because the genome of *M. truncatula* was fully sequenced and defense mechanisms against ARR had been previously evaluated (Badis et al. 2015; Jacquet et al. 2019). The cost of NGS can be reduced further by combining bulked segregant analysis with RNA-seq, in a process referred to as bulked segregant RNA-seq (BSR-seq). BSR-seq had been applied in maize, wheat and canola to identify differentially expressed gene and design resistance-specific markers (Liu et al. 2012; Yu et al. 2016; Hu et al. 2018; Wu et al. 2018), providing a new approach for transcriptomic analysis.

1.4 Hypothesis and objectives

The pea root rot complex represents a major constraint to field pea production in Canada and worldwide. The involvement of multiple pathogens, production of long-lasting survival structures, and absence of complete resistance make management of the PRRC difficult. The identification and development of pea germplasm with quantitative resistance to the root rot pathogens represents an effective and practical approach for disease management. Partial resistance to root rot and the associated QTL have been reported in other countries. In Canada, the pea cultivar '00-2067' showed partial resistance to this disease, but the genetic basis of this resistance has not been studied.

The objectives of this project were to: (1) identify the QTL associated with resistance to *A. euteiches* in '00-2067' using a high-throughput SNP array in an F_8 RIL population; (2) evaluate the efficiency and stability of the detected QTL; (3) evaluate the resistance to the *Fusarium* spp. implicated in root rot development; (4) identify the QTL related to resistance against these species; and (5) evaluate differentially expressed genes and predict the pathway(s) associated with resistance to *A. euteiches*. Thus, it was hypothesized that the cultivar '00-2067' contains polygenetic resistance to *A. euteiches*, which could be passed on to its progeny. An additional

hypothesis was that '00-2067' also carries broad tolerance to other pathogens involved in the PRRC, most notably *Fusarium* spp. The final hypothesis was that resistance to *A. euteiches* would be reflected in differential gene expression in resistant and susceptible pea, contributing to the different disease reactions. The detected disease-related genes may indicate defense mechanisms in a gene network associated with the interaction between field pea and *A. euteiches*.

Table 1.1. Summary of QTL identification studies on partial resistance to *Aphanomyces euteiches* and *Fusarium* spp.

Pathogen	Marker	#. QTL	Major QTL Chrom.	Population	Pop. Size	R Parental CV.	Reference
A. etueiches	classical markers, isozymes, STS markers and RAPDs	1	LGIV	RIL (F5-6)	45	MN313	Weeden et al. 2000
A. etueiches	AFLPs, RAPDs, SSRs, ISSRs, STSs	7	LGIV	RIL(F5,10)	127	PI 90-2079	Pilet-Nayol et al. 2002
A. etueiches	SSR, RAPD	7	LGIV	RIL(F5,10)	127	PI 180693 and PI 90-2131	Pilet-Nayol et al. 2005
A. etueiches	SSR, RAPD, RGA	135	LGII,III,IV,VI,VII	RIL(F8-9)	178	PI180693	Hamon et al. 2011
A. etueiches	SSR, RAPD	244	LGII,III,IV,VII	RIL(F8-10)	178	90–2131, 90–2079, Baccara, PI180693, Baccara and 552	Hamon et al. 2013
A. etueiches	SSR	7	LGIV,VII	NIL(F9)	157	RIL 847.50 and 552	Lavaud et al. 2015
A. etueiches	SNP	52	all chrom.	GWAS	175	90–2131, 552 and PI180693	Desgroux et al. 2016
A. etueiches	SNP	75	all chrom.	GWAS	266	90–2131, 552 and PI180693	Desgroux et al. 2018
F. solani	SSRs, RAPDs, morphological and gene-based markers	5	LGII	RIL (F8)	111	PI 557501	Coyne et al. 2015
F. solani	SNP	3	LGII	RIL (F8)	178	PI 180693	Coyne et al. 2019
F. avenaceum	SSR	1	LGVII	RIL (F8)	135	Carman	Feng et al. 2010
F. oxysporum	SSR, RAPDs and isozyme	2	LGIV	RIL (F7)	187	Shawnee	McPhee et al. 2012

Figure 1.1. Symptoms of the pea root rot complex. (A) Wilting and premature senescence of pea in a low-lying area of a field; (B) rotting of the stem and completely dead pea plants; and (C) infected pea roots (left) compared with healthy ones (right).

Chapter 2 Mapping QTL associated with partial resistance to Aphanomyces root rot in pea (*Pisum sativum* L.) using a 13.2K SNP array and SSR markers

2.1 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 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. 2018a, b). The soilborne oomycete *Aphanomyces euteiches* Drechs. is a dominant pathogen in the PRRC (Jones and Drechsler 1925). This pathogen is favored 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 fungicides Apron

FL (metalaxyl-M) and fosetyl-AL were reported to increase seedling emergence and delay the infection of field pea by *A. euteiches* in the early growth stages, but they failed to suppress ARR in the later stages, leading to yield losses (Oyarzun et al. 1990; Xue 2003). 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*. Chemical fungicides, however, are host non-specific and hence may affect beneficial microorganisms in the soil as well as pollinators. Furthermore, fungicides may leave residues on crops and persist in the environment. 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. 2015). 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 to 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). The availability of the pea reference genome (Kreplak et al. 2019) will facilitate the development of

different marker types for genotyping this important crop. 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.

2.2 Materials and methods

2.2.1 Plant Materials

One hundred and thirty-five F8- 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 and produced white flowers, a wrinkled seed coat, 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.

2.2.2 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). In brief, the oospore inoculum was generated in an oat broth as described by Papavizas and Ayers (1974). A preliminary inoculum density experiment was conducted to determine the concentration of inoculum that produced a differential reaction in the parents. This was done by first estimating the oospore concentration with a hemocytometer and adjusting the final concentration to 1×10^2, 1×10^3, 1×10^4, 1×10^5, and 1×10^6 oospores mL^{-1} with sterile deionized water. Based on this preliminary trial, an oospore concentration of 1×10^5 oospores mL^{-1} was selected for use in screening the parents as well as the RILs. The isolate *Ae-MRDC1* was re-isolated from infected root tips and was confirmed to be *A. euteiches* by PCR analysis with the species- specific primer set, 136F/211R (Vandemark et al. 2002).

2.2.3 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. (VSN International 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 when the plants were at the pods setting stage on July 22, 2015, and July 19, 2016. Disease assessment (DSF) was carried out on a 0-9 scale following Conner et al. (2013), where 0 = healthy root system and 9 = roots totally destroyed and rotten. Vigor (VF) was scored on a 0-4 scale, where 4 = completely healthy plant with no wilting and 0 = stunted plants with yellow leaves and dead stem and leaves (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.

2.2.4 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 × 7 × 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 soiless mix 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. Non-inoculated plants of the parental cultivars 'Reward' and '00-2067' were included as controls. 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 three times, referred to as greenhouse experiments 1 (GH1), 2 (GH2) and 3 (GH3).

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

2.2.5 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: $P_{ij} = \mu + G_i + R_j + e_{ij}$, where P_{ij} 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, G_i the i^{th} RIL effect or genetic effect, R_j the j^{th} replicate effect or blocking effect, and e_{ij} was the residual variance. The model for multiple station-years or greenhouse experiments was: $P_{ijk} = \mu + G_i + R_j + L_k + GL_{ijk} + e_{ijk}$, where P_{ijk} was the score of the i^{th} RIL located in the j^{th} replicate and k^{th} location, 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, L_k the k^{th} environment or location effect, GL_{ijk} the interaction of RIL effect and environment, and e_{ijk} 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. The power to detect a QTL for RILs of 135 individuals was determined using 'powercalc' in *R*/qtl for all the traits using the total data from the field and greenhouse, independently. The biological replicates, genetic variance and environmental variance were extracted and calculated from ANOVA.

2.2.6 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).

2.2.7 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).

2.2.8 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 (*Ae$_{MRDC1}$*, *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.Ae$_{MRDC1}$-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'.

2.2.9 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.Ae$_{MRDC1}$-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

2.3 Results

2.3.1 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 best linear unbiased predictors (BLUPs) of the experiments, are presented in Table 2.1 and Figure 2.1, respectively. The power to detect QTL given the 135 RIL population size used in this study was 0.99-1.00 for disease severity, vigor and foliar weight, and 0.67 for plant height.

2.3.2 Disease severity variation in the RIL population

The parental 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 indicated a significant effect of genotype on the RIL population ($P < 0.05$). The year-station effect for root rot severity in the greenhouse conditions and the disease nurseries was significant ($P < 0.05$) (Table 2.5). The correlation among individual experiments and BLUPs in the greenhouse and field was significant (r = 0.36-0.88, $P < 0.001$, Figure 2.1a). Therefore, the BLUPS were applied instead of the pooled data in the multiple model. The BLUPs of '00-2067' were calculated as 0.2 for the greenhouse data (DSGHB) and as 5.0 for the BLUPs field (DSFB) data. In the case of 'Reward', the BLUPs were 5.9 in the greenhouse and 8.2 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 2.1a), with low values for skewness and kurtosis (Table 2.1). The Shapiro-Wilk test of the disease severity data for normality was significant for all of the field and greenhouse experiments except for DSGH2. The genotypic effects of disease severity were significant ($P < 0.05$) in the greenhouse and field for the individual experiments. Entry mean-based heritability of disease severity was high, ranging from 60 to 92% (Table 2.6), which indicated a strong genetic effect of tolerance to *A. euteiches* that was transmitted from '00-2067' to individuals in RIL population.

2.3.3 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 SE 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 SE for DFGH1, DFGH2 and DFGH3, respectively, and 2.7 ± 1.2 SE and 1.1 ± 0.5 SE for DFF15 and DFF16. The BLUPs for '00-2067' and 'Reward' were 2.8 and 1.8 for the greenhouse experiments (DFGHB), and 11.3 and 0.6 for the field trials (DFFB), respectively. Significant differences between the parental cultivars were found with respect to foliar weight in the field and greenhouse experiments ($P < 0.05$). In addition, significant RIL genotype effects were found in the ANOVA of foliar weight in the greenhouse ($P < 0.05$) but not in the field experiment. Foliar weight of the RIL population in both the greenhouse and field trials had a continuous frequency distribution (Figure 2.1a). Based on the Shapiro-Wilk test, however, the data did not follow a normal distribution except for DFGH1 (Table 2.1). Foliar weight in the replicated greenhouse and field experiments was correlated significantly ($r = 0.50$-0.86, $P < 0.01$). Heritability of foliar weight was high, ranging from 67 to 94% (Table 2.6). This suggested that a significantly high percentage of genotypic effect ($P < 0.05$) was transmitted from the parents to the RIL population.

2.3.4 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 score for ‘00-2067’ was significantly higher relative 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 greenhouse and field data were continuous, but none were normally distributed (Table 2.1). The individual replications in the greenhouse and field were coincident based on the correlation analysis ($r = 0.33$-0.90, $P < 0.05$). A significant genotypic effect ($P < 0.05$) and high heritability (62-90%) were detected in the RIL population (Table 2.6). The BLUPs for vigor ratings for ‘00-2067’ were 4.2 and 4.0 in the field and greenhouse, respectively, while for ‘Reward’, the BLUPs were 2.6 and 0.7, respectively.

2.3.5 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 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 SE 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 SE for HGH1, HGH2 and HGH3, respectively. The BLUPs for plant height (HGHB) for ‘00-2067’ were 183.7 mm, and for ‘Reward’ it was 130.1 mm. 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 (Table 2.1). 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 94% for HGH1, HGH2, HGH3 and HGHB, respectively. (Table 2.6).

2.3.6 Correlation analysis among traits

All of the traits in different environments were significantly and positively correlated with

each other ($0.33 < r < 0.95$, $P < 0.001$). Correlation analysis among variables indicated that all the traits were significantly correlated with each other in the individual experiments and in the BLUPs ($0.31 < r < 0.90$, $P < 0.001$), except for plant height, which was only coincidently correlated with vigor ($0.18 < r < 0.38$, $P < 0.05$) and dry foliar weight ($0.59 < r < 0.71$, $P < 0.001$). 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 (Figure 2.1b).

2.3.7 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 2.2). 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 from this study corresponded to linkage group *I* (Ia and Ib) of the pea genome by Tayeh et al. (2015) and to chromosome 2 by Neumann et al. (2002) (Table 2.2). Linkage groups 3, 4, 5, 8 and 9 from this study corresponded to the linkage groups II, III, IV, VI, and VII, respectively, reported by Tayeh et al. (2015) and to chromosomes 6, 5, 4, 1 and 7, respectively, reported by Neumann et al. (2002) (Table 2.2). Linkage groups 6 and 7 from this study corresponded to linkage group V and chromosome 3 of the pea genome reported by Tayeh et al. (2015) and Neumann et al. (2002), respectively (Table 2.2). The relationships between the linkage groups identified in this study and the pea reference genome reported by Kreplak et al. (2019) are provided in Table 2.2. The number of markers per chromosome in this study ranged from 103 in linkage group 8 (chrom 1/LG VI of the pea genome) to 334 in linkage group 9 (chrom 7/LG VII of the pea genome). The length of the seven chromosomes ranged from 155.6 cM in linkage group 8 (chrom 1/LG VI of the pea genome) to 395.7 cM in linkage group 4 (chrom 5/ LG III of the pea genome) and spanned a total length of 1704.1 cM. The marker density per cM ranged from 1.1 to 2.4 and averaged 1.8 markers per cM

(Table 2.2). For convenience and ease of comparison with previous studies, we will use a modified linkage group designation of Tayeh et al. (2015) and the pea chromosome nomenclature of Neumann et al. (2002) in the rest of this paper.

2.3.8 Additive-effect QTL analysis

One thousand five hundred and seventy-seven (1577) of the 2999 markers (Table 2.2) mapped to the same position as other markers and were excluded from the QTL analysis. Therefore, only 1422 (10.5%) of the initial 13,426 (13,204 SNP + 222 SS) markers used to genotype the 135 RIL population were used for QTL analysis. Collectively, 27 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. 2012). Based on the *R*2 values, 8 of these QTL were major-effect QTL, 13 were moderate-effect QTL and 6 were minor-effect QTL. Ten of the 27 QTL were detected consistently in the greenhouse and field experiments, and could be classified as stable, while the remaining 17 were year- or experiment-specific QTL detected in only one environment (BLUPs data not counted).

2.3.9 QTL for tolerance to ARR

Eight putative QTL for partial resistance or tolerance to *A. euteiches* isolate *Ae-MRDC1* were detected by the CIM (Table 2.3). The QTL *Ae$_{MRCD1}$-Ps2.1*, detected in the 2015 field experiment (DSF15) and in the BLUPs of the GH (DSGHB) data, and a second QTL *Ae$_{MRCD1}$-Ps2.2* detected in the 2016 field (DSF16) experiment, explained 14.1-14.5% and 13.8% of the total variation in ARR severity, respectively. The QTL *Ae$_{MRCD1}$-Ps3.1*, detected in GH experiment 2 (DSGH2) and the DSGHB data, explained 47.7% and 28.3% of the phenotypic variance, respectively. The QTL *Ae$_{MRCD1}$-Ps3.2*, detected in the BLUPs of the field data (DSFB), accounted for 32.3% of the phenotypic variance. The QTL *Ae$_{MRCD1}$-Ps4.1*, detected in DSF16 and DSGH1 as well as in the BLUPs of the field data (DSFB), accounted for 17.1%, 18.6% and 15.4% of the phenotypic variance, respectively. A second QTL *Ae$_{MRCD1}$-Ps4.2*, located very close to *Ae$_{MRCD1}$-Ps4.1*, detected in DSGH2 and DSGH3 as well as in the BLUPs of the greenhouse data (DSGHB),

accounted for 18.2%, 12.5% and 17.4% of the phenotypic variance, respectively. Thus, these two QTL, which are in close proximity to each other, were detected in four of the five environments. *Ae$_{MRCD1}$-Ps7.1* and *Ae$_{MRCD1}$-Ps7.2*, detected in the DSF16 experiment, explained 17.2% and 11.2% of the phenotypic variance, respectively. In addition, *Ae$_{MRCD1}$-Ps7.2* was detected in the BLUPs of the field data (DSFB) and accounted for 7.7% of the phenotypic variance (Table 2.3).

The QTL *Ae$_{MRCD1}$-Ps2.1* mapped to the top segment of LG II (chrom 6) (peak marker, 19.3 cM) flanked by the SNP markers PsCam043049_27080_440 and PsCam000084_71_191. Similarly, *Ae$_{MRCD1}$-Ps2.2* also mapped to the top segment (38.3 cM) of LG II (chrom 6), flanked by the SNP markers PsCam031050_18310_2958 and PsCam045443_29114_1358 (Figure 2.2a). *Ae$_{MRCD1}$- Ps3.1* mapped to the middle segment (167-172 cM) of LG III (chrom 5), flanked by the SNP markers PsCam044942_28684_3048 and PsCam036430_21570_962 (Figure 2.2b). *Ae$_{MRCD1}$-Ps3.2* mapped to the middle segment (292.6 cM) of LG III (chrom 5), flanked by the SNP marker PsCam020937_11699_2576 and the SSR marker AA5 (Figure 2.2b). *Ae$_{MRCD1}$-Ps4.1* mapped to the top segment (39.6-41.2 cM) of LG IV (chrom 4), flanked by the SNP markers PsCam035653_20827_1413 and PsCam027250_15918_2181 (Figure 2.2c). *Ae$_{MRCD1}$- Ps4.2* mapped to the middle segment (51.8-54.6 cM) of LG IV (chrom 4), flanked by the SNP markers PsCam000402_353_679 and PsCam001381_1152_437 (Figure 2.2c). *Ae$_{MRCD1}$-Ps7.1* mapped to the middle segment (124.1 cM) of LG VII (chrom 7), flanked by the SNP markers PsCam058653_39030_117 and PsCam060132_40119_811, while *Ae$_{MRCD1}$-Ps7.2* mapped to the bottom segment (211.8-212.5 cM) of LG VII (chrom 7), flanked by the SNP markers PsCam033614_19198_1651 and PsCam024843_14133_904 (Figure 2.2d).

The additive effects for *Ae$_{MRCD1}$-Ps3.1, Ae$_{MRCD1}$-Ps3.2*, *Ae$_{MRCD1}$-Ps-C4.1*, *Ae$_{MRCD1}$-Ps4.2* and *Ae$_{MRCD1}$-Ps7.2* had negative values. This indicated that the favorable alleles for partial resistance originated from '00-2067'. In contrast, the additive effects for *Ae$_{MRCD1}$-Ps2.1*, *Ae$_{MRCD1}$-Ps2.2* and *Ae$_{MRCD1}$-Ps7.1* had positive values, indicating that the alleles originated from 'Reward'.

2.3.10 QTL for foliar weight

Seven putative QTL were detected by the CIM for foliar weight in the RIL population

inoculated with *A. euteiches* isolate *Ae-$_{MRDC1}$* (Table 2.3). *Fwt-Ps2.1* detected in the 2016 field (DFF16) experiment and the BLUPs of the field data (DFFB) data explained 5.3% and 6.5%, respectively, of the total variation in foliar weight. *Fwt-Ps2.2*, detected in only the DFF16 experiment, explained 2.6% of the phenotypic variance. The QTL *Fwt-Ps4.1*, detected in the DFGH1 experiment, explained 14.6% of the phenotypic variance. *Fwt-Ps4.2*, detected in DFF16 and DFGH2 experiments as well as the DFGHB data, explained 32.2, 11.8, and 22.8% of the phenotypic variance, respectively. Two QTL, *Fwt-Ps6.1* and *Fwt-Ps6.2* detected in the DFF16 experiment, explained 2.9% and 13.3% of the phenotypic variance, respectively. The QTL *Fwt-Ps7.1*, detected in the DFGH1 and DFGH3 experiments, accounted for 9.0% and 14.8% of the phenotypic variance, respectively.

The QTL *Fwt-Ps2.1* and *Fwt-Ps2.2* mapped to the top (44.5 cM) and middle (91.7 cM) segments of LG II (chrom 6), respectively. The SNP markers PsCam020818_11602_1430 and PsCam050370_32957_642 flanked *Fwt-Ps2.1*, while PsCam042179_26280_4473 and PsCam011350_7726_1258 flanked *Fwt-Ps2.2* (Figure 2.3a). *Fwt- Ps4.1* and *Fwt-Ps4.2* mapped to the top (27.0; 38.0-51.8 cM) segment of LG IV (chrom 4). The SNP markers PsCam054029_35722_104 and PsCam037549_22628_1642 flanked *Fwt-Ps4.1*, while PsCam054029_357722_104 and PsCam042892_26931_689 flanked *Fwt-Ps4.2* (Figure 2.3b). *Fwt-Ps6.1* mapped to the tip (0.4 cM) of LG VI (chrom 1), while *Fwt-Ps6.2* mapped to the middle (69.4 cM) segment of LG VI (chrom 1). The SNP markers PsCam000459_401_464 and PsCam001884_1539_754 flanked *Fwt-Ps6.1*, while PsCam011542_7868_781 and PsCam037575_22653_1339 flanked *Fwt-Ps6.2* (Figure 2.3c). *Fwt-Ps7.1* mapped to the (127.3-142.3 cM) segment of LG VII (chrom 7). The SNP markers PsCam006867_5111_92 and PsCam039434_24372_625 flanked *Fwt-Ps7.1* (Figure 2.3d).

The additive effects for *Fwt-Ps2.2*, *Fwt-Ps4.1*, *Fwt-Ps4.2*, *Fwt-Ps6.2* and *Fwt-Ps7.1* had positive values. This indicated that the favorable alleles for foliar weight originated from '00-2067'. In contrast, the additive effects for *Fwt-Ps2.1* and *Fwt-Ps6.1* had negative values, indicating that the alleles originated from 'Reward'.

2.3.11 QTL for vigor

Seven putative QTL were detected via CIM for vigor in the RIL population inoculated with *A. euteiches* isolate *Ae-* $_{MRDC1}$ (Table 2.3). The QTL *Vig-Ps1.1* detected in the 2015 field (VF15) and 2016 field (VF16) experiments explained 12.1% and 10.7% of the phenotypic variance, respectively. *Vig-Ps2.1*, detected in the VF15 experiment, explained 15.6% of the phenotypic variance. *Vig-Ps2.2*, detected in the 2016 field (VF16) experiment and BLUPs of the field (VFB) data, explained 8.7% and 8.3% of the phenotypic variance, respectively. *Vig-Ps3.1* was highly stable, accounting for 41.09% of the phenotypic variance in the VF16 experiment, 16.1% and 28.0% of the variance in two GH experiments (VGH1 and VGH2, respectively), and 18.3% of the phenotypic variance in the BLUPs of the greenhouse (VGHB) data. *Vig-Ps3.2*, detected in the VFB data, explained 29.3% of the phenotypic variance. *Vig-Ps4.1*, detected in the VG15 experiment and the VFB of the field data, explained 24.0% and 15.9% of the phenotypic variance, respectively. *Vig-Ps4.2* (located distal to *Vig-Ps4.1*) was highly stable across the VF16, VGH2, VGH3 and VFB experiments, explaining 29.2, 20.3, 26.3 and 14.9% of the phenotypic variance, respectively (Table 2.3).

Vig-Ps1.1, which was mapped to the top-to-middle segment (49.9 cM) of LG I (chrom 2), was flanked by the SNP markers PsCam048937_31589_2232 and PsCam003924_2990_265 (Figure 2.4a). *Vig-Ps2.1* and *Vig- Ps2.2* mapped to the top segment (17.9, 38.3 cM) of LG II (chrom 6), with the former flanked by the SNP markers PsCam043049_27080_440 and PsCam000362_321_595 and the latter flanked by the SNP markers PsCam031050_18310_2958 and PsCam002058_1676_641 (Figure 2.4b). *Vig-Ps3.1* mapped to the middle segment (165.0-188.3 cM) of LG III (chrom 5) and was flanked by the SNP markers PsCam005343_4052_245 and PsCam035416_20603_89, while *Vig-Ps3.2* 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 2.4c). *Vig-Ps4.1* and *Vig-Ps4.2* mapped to the top-to-middle segment (41.2-44.5 cM and 51.8-54.6 cM, respectively) of LG IV (chrom 4). The SNP markers PsCam048119_30866_192 and PsCam010902_7362_452 flanked *Vig-Ps4.1*, while PsCam001086_922_203 and

PsCam001381_1152_437 flanked *Vig-Ps4.2* (Figure 2.4d).

The additive effects for *Vig-Ps3.1*, *Vig-Ps3.2*, *Vig-Ps4.1* and *Vig-PsC4.2* had positive values, indicating that the favorable alleles for vigor originated from '00-2067'. In contrast, the additive effects for *Vig-Ps1.1*, *Vig-Ps2.1* and *Vig-Ps2.2* had negative values, which indicated that the alleles originated from 'Reward'.

2.3.12 QTL for plant height

Five putative QTL for plant height were detected by CIM in the RIL population inoculated with *A. euteiches* isolate *Ae-$_{MRDC1}$* (Table 2.3). Three of the five QTL, *Hgt-Ps3.1*, *Hgt-Ps4.1* and *Hgt-Ps7.1*, were highly stable across at least two of the GH experiments (HGH1, HGH2 and HGH3) as well as in the BLUPs of the greenhouse data (HGHB). The percentage of phenotypic variance explained by *Hgt- Ps3.1*, *Hgt-Ps4.1* and *Hgt-Ps7.1* ranged from 13.4 to 18.7%, 10.0-18.6% and 15.8-24.9%, respectively. The QTL *Hgt- Ps3.2* detected in the HGH2 experiment and HGHB data explained 14.6% and 17.1%, respectively, of the phenotypic variance. The QTL *Hgt-Ps5.1* detected in the HGH1 experiment explained 6.3% of the phenotypic variance (Table 2.3).

Hgt-Ps3.1 and *Hgt-Ps3.2* mapped to the bottom segment (287.6 cM and 304.3 cM, respectively) of LG III (chrom 5). The former was flanked by the SNP marker PsCam020937_11699_2576 and the SSR marker AA5, while the latter was flanked by the SSR marker AA5 and the SNP marker PsCam036163_21311_1095 (Figure 2.5a). *Hgt-Ps4.1* and *Hgt-Ps5.1* mapped to the top segments of LG IV (13.7 cM; chrom 4) and LG V (1.1 cM; chrom 3), respectively. *Hgt-Ps4.1* was flanked by the SNP markers PsCam000228_198_1085 and PsCam037026_22136_167 (Figure 2.5b), while *Hgt-Ps5.1* was flanked by the SNP markers PsCam017782_10917_295 and PsCam005127_3886_1505 (Figure 2.5c). *Hgt-7.1* mapped to the middle segment (124.3-132.3 cM) of LG VII (chrom 7), and was flanked by the SNP markers PsCam021891_12310_347 and PsCam006867_5111_92 (Figure 2.5d).

The additive effects for *Hgt-Ps4.1* and *Hgt-Ps7.1* had positive values. This indicated that the favorable alleles for height originated from '00-2067'. On the other hand, the additive effects for *Hgt-Ps3.*1, *Hgt-Ps3.2* and *Hgt-Ps5.1* had negative values, indicating that the alleles originated from

'Reward'.

2.3.13 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, 17-27, 17-22 and 18-24 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 BLUPs data, the number of putative digenic interactions detected was 20, 24, 25, 18, 21, 29 and 28 for DSFB, DSGHB, DFFB, DFGHB, VFB, VGHB and HGHB, respectively (Figure 2.6). Of the 165 digenic interactions detected in the BLUPs analysis, 7 QTL pairs had 7.5% ≤ PVE ≤ 15% and 158 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 2.4.

Markers for 24 of the 27 major- and moderate-effect epistatic QTL (Table 2.4) were also linked to 17 additive-effect QTL (*Ae$_{MRDC1}$-Ps2.1*, *Ae$_{MRDC1}$-Ps3.1*, *Ae$_{MRDC1}$-Ps4.1*, *Ae$_{MRDC1}$-Ps7.1*, *Ae$_{MRDC1}$-Ps7.2*, *FwtPs-4.1*, *FwtPs-4.2*, *FwtPs-6.2*, *FwtPs-7.1, VigPs-1.1*, *VigPs-2.1*, *VigPs-3.1*, *VigPs-4.1*, *VigPs-4.2*, *Hgt-3.2*, *HgtP4.1*, and *VigPs-7.1*) identified in this study. The most significant QTL × QTL interaction, *E.Ae$_{MRCD1}$-Ps7*, involved markers linked to five major additive-effect QTL for root rot severity (*Ae$_{MRDC1}$- Ps4.1, R^2* = 15.4 - 18.6%), foliar weight (*FwtPs-4.2*, R^2 = 11.8 - 32.2%), vigor (*VigPs-4.1*, R^2 = 15.9 - 24.0%; *VigPs-4.2*, R^2 = 20.3 - 29.2%) and height (*HgtPs-4.1*, R^2 = 10.0 - 18.6%), as well as the moderate additive effect QTL *FwtPs-4.1* (R^2 = 14.6%). The second important QTL × QTL interaction, *E.Vig-Ps2*, involved markers linked to five major additive-effect QTL, *Ae$_{MRDC1}$-Ps3.1* (R^2 = 28.3 - 47.7%), *Ae$_{MRDC1}$-Ps4.1* (R^2 = 15.4 - 18.6%), *FwtPs-4.2* (R^2 = 11.8 - 32.2%) , *Vig-Ps3 .1* (R^2 = 16.1 - 41.1%), and *Vig-Ps4.2* (*R*2 = 20.3 - 29.2%). The third important QTL × QTL interaction, *E.Ae$_{MRCD1}$- Ps11*, involved markers linked to four major additive-effect QTL, *Ae$_{MRDC1}$-Ps3.1* (R^2 = 28.3 - 47.7%), *Ae$_{MRDC1}$-Ps4.1* (R^2 = 15.4 - 18.6%), *Vig-Ps3.1* (R^2 = 16.1 - 41.1%), and *Vig-Ps4.2* (R^2 = 20.3 - 29.2%) and the moderate additive effect

QTL (*Ps.Fwt-4.1*, R^2 = 14.6%). The fourth important QTL × QTL interaction, *E.Ae$_{MRCD1}$-Ps1*, involved markers linked to three major additive-effect QTL, *Ae$_{MRDC1}$-Ps3.1* (R^2 = 28.3 - 47.7%), *Vig-Ps2.1* (R^2 = 15.6%), and *Vig-Ps3.1* (R^2 = 16.1 - 41.2%) and the moderate additive-effect QTL *Ae$_{MRDC1}$-Ps2.1* (R^2 = 14.1-14.5%). Fourteen QTL × QTL interactions, *E.Ae$_{MRCD1}$-Ps4*, *E.Ae$_{MRCD1}$-Ps5*, *E.Ae$_{MRCD1}$- Ps6*, *E.Ae$_{MRCD1}$-Ps9*, *E.Ae$_{MRCD1}$-Ps10*, *E.Fwt-Ps2, E.Fwt- Ps4*, *E.Vig-Ps3*, *E.Vig-Ps4*, *E.Vig-Ps5*, *E.Vig-Ps6*, *E.Vig-Ps7*, *E.Vig-Ps8* and *E.Vig-Ps9*, involved markers linked to at least two major additive-effect QTL, *Ae$_{MRDC1}$-Ps3.1* (R^2 = 28.3 - 47.7%) and *Vig-Ps3.1* (R^2 = 16.1 - 41.1%). The QTL × QTL interaction *E.Hgt-Ps2* involved markers linked to the major additive-effect QTL *Ae$_{MRDC1}$-Ps7.1* (R^2 = 17.2%) and *Hgt-Ps7.1* (R^2 = 15.8 - 24.9%), and the moderate additive effect QTL *Fwt-Ps7.1* (R^2 = 9.0 - 14.8%). Five other QTL × QTL interactions, *E.Ae$_{MRCD1}$-Ps3*, *E.Ae$_{MRCD1}$-Ps8*, *E.Fwt-Ps1, E.Fwt-Ps3* and *E.Vig-Ps1*, involved markers linked to either one (*Fwt-Ps4.1* or *Hgt-Ps3.2*) major additive effect or one (*Fwt-Ps6.2*) minor additive-effect QTL. Markers linked to three QTL × QTL interactions, *E.Ae$_{MRDC1}$-Ps2*, *E.Hgt-Ps1* and *E.Hgt-Ps3*, were not associated with any of the additive-effect QTL (Table 2.4).

2.3.14 Putative functions of proteins encoded by identified sequences

The sequences identified in this study matched entries in the Pulse Crop Database that included pathogenesis-related proteins, cellulose synthase, SBP domain, F-box associated

domain, phospholipase X-box domain, phospholipase-like protein (PEARLI 4), protein tyrosine kinase, UDP-glycosyl transferase, methyl transferase domain, WRKY DNAbinding domain, pectin acetylesterase, ABC transporter protein, PPR repeat family, jacalin-like lectin domain, Barwin- related endoglucanase, zinc finger SWIM-type protein, protein phosphatase 2C, and gibberellin regulated protein. These proteins are associated with plant defense responses and regulation, cell wall components and properties, biological processes, as well as to the response to abiotic and biotic stresses.

2.4 Discussion

This study evaluated tolerance or partial resistance to ARR in an F8 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 single experiments and the BLUPs data for all traits in the field trials or greenhouse experiments were continuous, but deviated from normality with various levels of skewness and kurtosis, which is not unusual for field disease data (Eskridge 1995; Feng et al. 2011; 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.

Mohan et al. (1997) and Collard et al. (2005) reported that for a preliminary mapping study, a population size of 50-250 individuals was sufficient to reduce genotyping costs. Therefore, it was essential to determine the effective population size to obtain enough power for this analysis. Power analysis confirmed the sufficiency (close to 1) of the 135 RILs used in this study for disease severity, dry foliar weight and vigor. In contrast, the power of plant height (0.67) in the greenhouse was lower than the 0.8 threshold (Hu and Xu 2018; Kim 2016; Serdar et al. 2021). The small difference between parents for plant height indicated that this trait was not affected by ARR, but rather reflected the genetics of the field pea, which was also indicated by correlation analysis. The number of individuals included this study was within the range of 111-178 used by Pilet-Nayel et al. (2002, 2005) and Hamon et al. (2011, 2013) for the detection of QTL associated with ARR resistance in field pea.

Transgressive segregation, in which some lines were more resistant or susceptible than the resistant and susceptible parents, was observed 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 that resistance genes in the parents residing on different linkage groups could account for the higher levels of tolerance exhibited by some of the RILs. The RILs exhibiting greater tolerance to ARR than the parents could be used in genetic crosses to stack

the resistance genes, and the potential of the developed lines for various breeding programs should be exploited. Furthermore, sequence comparison between the tolerant parent and the RILs that showed greater tolerance could enable the identification of tightly linked markers for marker assisted breeding.

Although the G × E interaction for all traits were significant, moderate to high correlation coefficients were observed for all the traits between the field and greenhouse, as well as among individual experiments in the field and greenhouse and in the BLUPs data. High heritability of traits in each single field or greenhouse experiment, as well as in the BLUPs data, also confirmed the significant genetic effects on ARR tolerance in RILs. We observed a high correlation for disease severity and other traits in the field trials and greenhouse experiments (Figure 2.1). Previous studies in chickpea (*Cicer arietinum*) (Johansen et al. 1994) and snap bean (*Phaseolus vulgaris*) (Navarro et al. 2008) indicated 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, foliar weight and vigor (Table 2.4). The observation that about 81.5% (22 out of 27) 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 7.4% (2 out of 27) 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 are consistent with the findings of Conner et al. (2013), who suggested that ARR affected foliar weight and plant vigor but not plant height.

In the QTL mapping, genomic regions corresponding to 27 QTL for root rot severity, two disease-related criteria (foliar weight and vigor) and one agronomic trait (plant height) were identified. The largest (R^2 of 28.3-47.7%) major-effect QTL, *$Ae_{MRCD1}Ps$-3.1*, for resistance to *A. euteiches* identified in this study was found on LG III effect QTL, *$Ae_{MRCD1}Ps$-4.1,* was located on LG IV (chrom 4) (Table 2.3). The QTL on LG IV (chrom 4) was the most stable, since it was detected in four of the five experiments, while the QTL on LG III (chrom 5) was detected in only one experiment (BLUPs data not counted). The largest major effect QTL detected by Pilet-Nayel

et al. (2002, 2005), *APh1* (R^2 of up to 47%), was also located on LG IV (chrom 4). Weeden (2000) also reported that a gene influencing tolerance to common root rot in pea was located on LG IV (chrom 4). In contrast, the third largest major-effect QTL (*AePs4.1*) detected by Hamon et al. (2011) was located on LG IV (chrom 4). The second major-effect QTL detected by Hamon et al. (2011) (*Ae-Ps3.1*; R^2 of up to 29.9%) was located on LG III (chrom 5). Hamon et al. (2011) detected *Ae-Ps3.1* consistently in multiple experiments, while in this study *Ae$_{MRCD1}$Ps-3.1* was identified in only one of the greenhouse experiments.

The QTL for resistance to *A. euteiches* isolate *Ae-$_{MRDC1}$* located on LG II (chrom 6), *Ae$_{MRCD1}$Ps-2.1*, was found to be a moderate-effect QTL with R^2 = 14.1 - 14.5%, while those on LG VII (chrom 7), *Ae$_{MRCD1}$Ps7.1* and *Ae$_{MRCD1}$Ps-7.2*, were found to be minor-effect and moderate-effect QTL, respectively, with R^2 values ranging from 7.7 to 17.2%. The QTL reported on LG II (chrom 6) and LG VII (chrom 7) by Hamon et al. (2011) were found to be a combination of minor-effect and 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 LG VII (chrom 7) 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 LG VII (chrom 7) for disease-related criteria, namely aboveground index and root weight loss. Similar to the findings of Pilet-Nayel et al. (2005), the most stable and consistently detected QTL with the largest R^2 for aboveground disease indices (foliar weight and vigor) were located on LG IV (chrom 4), 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), appear to be on similar chromosomes, despite the different pedigrees of the parents.

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 of 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 peak genomic regions corresponding to Ae_{MRCD1}*Ps-4.1*, *Fwt-Ps4.1* and *Vig-Ps4.1* and Ae_{MRCD1}*Ps-4.2*, *Fwt-Ps4.2* and *Vig-Ps4.2* were within ≈ 3.0-5.0 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 entire 38.0-58.0 cM region on LG IV (chrom 4) may be an important breeding objective.

Based on our linkage map, the SNP marker s PsCam03754922628_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

the 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). Similarly, the genomic region corresponding to *Ae$_{MRCD1}$Ps-3.1* and *Vig-Ps3.1* on LG III (chrom 5), associated with partial resistance to ARR and vigor, respectively, is important for breeding. The peak region, however, was vast (5.0-33.0 cM) and contained fewer markers. As such, more markers need to be developed to fine map this genomic region as well as to identify candidate ARR resistance genes for future cloning and gene functional analysis. One approach could be to sequence the parents and each individual RIL, although genotyping costs may be prohibitively high for such a large number of genotypes. Alternatively, resequencing of important genomic regions, such the identified regions on LG IV (chrom 4) and LG III (chrom 5), would be more cost-effective. Recently, bulk segregant RNA-sequencing (BSR-seq) technology has emerged as a novel tool for the study of disease resistance and other traits in important crops (Liu et al. 2012; Yu et al. 2016; Hu et al. 2019; Wu et al. 2018a, b). BSR-seq in pea could help to identify the genes involved in various biological processes, regulation and development, as well as those involved in defense.

Previous studies evaluated partial resistance mainly by using disease severity (Pilet-Nayel et al. 2002, 2005; Hamon et al. 2011, 2013; Lavaud et al. 2015; Desgroux et al. 2016) and parameters related to underground losses in pea (Pilet-Nayel et al. 2002, 2005; Hamon et al. 2011, 2013; Desgroux et al. 2016). While the trait names and scoring scales were different, the vigor in our study was similar to above ground index (AGI) (Pilet-Nayel et al. 2002, 2005) or aerial decline index (ADI) (Hamon et al. 2011, 2013; Desgroux et al. 2016). Pilet-Nayel et al. (2002, 2005) also used the trait percentage of dried weight losses (DWL), while we applied dry foliar weight to indicate the effect of ARR on aboveground biomass. Thus, the parameters measured in this study were consistent with those used in the aforementioned studies.

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.

Table 2.1. 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 best linear unbiased predictors (BLUPs) of the greenhouse and the field experiments.

	'00-2067'	'Reward'		RIL population				
Abbrev.	Mean	Mean	P value	Mean	G effect	Skewness	Kurtosis	Shapiro test (*P*)
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
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
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
DSGHB	0.2	5.9		4.4 ±1.8		-0.3	-0.5	3.01E-02
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
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
DSFB	5.0	8.2		7.2 ± 1.4		0.1	-1.1	5.04E-05
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
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
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
DFGHB	2.8	1.8		2.8 ± 1.5		0.6	0.3	4.67E-02
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
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
DFFB	11.3	0.6		4.3± 4.1		1.0	0.2	0.00E+00
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
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
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
VGHB	4.0	2.6		3.1 ± 0.8		-0.1	-0.9	2.13E-03
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
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
VFB	4.2	0.7		2.8 ± 1.1		0.2	-0.7	5.39E-04
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
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
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
HGHB	183.7	130.1		187.5 ± 135.8		0.6	-0.5	3.0E-02

Table 2.2. 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'

LG	Relationship with previously reported nomenclature			# markers for QTL mapping				length/ (cM)	Marker density/ cM
	Chromosome[α]	Linkage group[β]	Pseudomolecules[γ]	SNP	SSR	Total	Bins		
1	Chrom 2	LG I	Chrom2LG1	225	3	228	173	205.5	1.1
2	Chrom 2	LG I	Chrom2LG1	59	0	59	32	21.2	2.8
3	Chrom 6	LG II	Chrom6LG2	489	5	494	272	251.6	2.0
4	Chrom 5	LG III	Chrom5LG3	525	7	532	197	395.7	1.3
5	Chrom 4	LG IV	Chrom4LG4	497	2	499	151	211.3	2.4
6	Chrom 3	LG V	Chrom3LG5	166	0	166	55	69.7	2.4
7	Chrom 3	LG V	Chrom3LG5	140	1	141	105	95.8	1.5
8	Chrom 1	LG VI	Chrom1LG6	263	0	263	103	155.6	1.7
9	Chrom 7	LG VII	Chrom7LG7	614	3	617	334	298.5	2.1
			Total or Average	2978	21	2999	1422	1704.9	1.8

[α] Pea chromosomes named according to Neumann et al. (2002), [β] Pea linkage groups named according to Tayeh et al. (2015) and [γ]pseudomolecule labels in the pea genome assembly v1a named according to Kreplak et al. (2019).

Table 2.3. 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 abbrev.	LG	Chrom$^{\alpha}$ / LG$^{\beta}$	Peak (cM)	Confidence interval(cM)	Left Marker	LOD	Additive	R^2 (%)
Ae_{MRCD1}Ps-2.1	DSF15	3	Chrom6/ LGII	19.3	15.7-27.0	PsCam043049_27080_440	4.5	0.4132	14.45
	DSGHB	3	Chrom6/LGII	19.3	14.3-26.9	PsCam043049_27080_440	4.7	0.6821	14.05
Ae_{MRCD1}Ps-2.2	DSF16	3	Chrom6/LGII	38.3	31.5-46.6	PsCam031050_18310_2958	4.3	0.3828	13.79
Ae_{MRCD1}Ps-3.1	DSGH2	4	Chrom5/LGIII	167.0	154.1-194.4	PsCam044942_28684_3048	5.4	-1.1474	47.70
	DSGHB	4	Chrom5/LGIII	172.0	155.8-195.9	PsCam044942_28684_3048	3.6	-1.0084	28.33
Ae_{MRCD1}Ps-3.2	DSFB	4	Chrom5/LGIII	292.6	287.4-297.6	PsCam020937_11699_2576	4.6	-0.8491	32.29
Ae_{MRCD1}Ps-4.1	DSF16	5	Chrom4/LGIV	41.2	38.2-42.5	PsCam037549_22628_1642	6.3	-0.4937	17.11
	DSFB	5	Chrom4/LGIV	41.2	38.7-42.5	PsCam037549_22628_1642	6.4	-0.7381	18.55
	DSGH1	5	Chrom4/LGIV	39.6	36.8-42.7	PsCam035653_20827_1413	4.6	-0.6338	15.40
Ae_{MRCD1}Ps-4.2	DSGH2	5	Chrom4/LGIV	51.8	47.5-55.3	PsCam000402_353_679	6.1	-0.8287	18.15
	DSGH3	5	Chrom4/LGIV	54.6	53.0-60.9	PsCam043430_27439_1668	4.5	-0.5618	12.45
	DSGHB	5	Chrom4/LGIV	54.6	53.8-58.4	PsCam043430_27439_1668	7.6	-0.8844	17.40
Ae_{MRCD1}Ps-7.1	DSF16	9	Chrom7/LGVII	124.1	117.0-136.4	PsCam058653_39030_117	5.7	0.5293	17.23
Ae_{MRCD1}Ps-7.2	DSF16	9	Chrom7/LGVII	211.8	209.7-215.5	PsCam033614_19198_1651	5.2	-0.3791	11.18
	DSFB	9	Chrom7/LGVII	212.5	211.5-216.4	PsCam033614_19198_1651	3.2	-0.4423	7.66
Fwt-Ps2.1	DFF16	3	Chrom6/LGII	44.5	35.1-47.6	PsCam020818_11602_1430	4.8	-0.0356	5.29
	DFFB	3	Chrom6/LGII	44.5	39.2-47.6	PsCam005315_4032_1360	4.7	-0.7931	6.51
Fwt-Ps2.2	DFF16	3	Chrom6/LGII	91.7	90.1-91.9	PsCam042179_26280_4473	4.9	0.0199	2.56
Fwt-Ps4.1	DFGH1	5	Chrom4/LGIV	27.0	24.1-33.1	PsCam054029_35722_104	8.6	0.3999	14.64
Fwt-Ps4.2	DFF16	5	Chrom4/LGIV	51.8	50.1-55.5	PsCam000015_11_1425	10.9	0.1172	32.15
	DFFB	5	Chrom4/LGIV	51.8	50.1-55.7	PsCam000015_11_1425	6.3	2.0086	22.80
	DFGH2	5	Chrom4/LGIV	38.0	28.8-42.3	PsCam054029_35722_104	3.4	0.3451	11.79
Fwt-Ps6.1	DFF16	8	Chrom1/LGVI	0.4	0.0-3.9	PsCam000459_401_464	4.4	-0.0300	2.92
Fwt-Ps6.2	DFF16	8	Chrom1/LGVI	69.4	56.9-78.2	PsCam011542_7868_781	4.0	0.0890	13.29
Ps.Fwt-7.1	DFGH1	9	Chrom7/LGVII	127.3	117.9-135.5	PsCam047459_30486_180	3.3	0.5694	8.96
	DFGH3	9	Chrom7/LGVII	142.3	135.5-148.6	PsCam006867_5111_92	4.6	0.3582	14.77

Table 2.3 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 abbrev.	LG	Chrom[α] /LG[β]	Peak (cM)	Confidence interval (cM)	Left Marker	LOD	Additive	R^2 (%)
Vig-Ps1.1	VF15	1	Chrom2/LGI	49.9	47.9-52.0	PsCam048937_31589_2232	4.7	-0.5847	12.13
	VF16	1	Chrom2/LGI	49.9	49.1-53.8	PsCam027866_16405_263	3.4	-0.4929	10.69
Vig-Ps2.1	VF15	3	Chrom6/LGII	17.9	10.7-20.0	PsCam043049_27080_440	3.8	-0.4447	15.55
Vig-Ps2.2	VF16	3	Chrom6/LGII	38.3	31.5-49.0	PsCam031050_18310_2958	3.6	-0.2505	8.68
	VFB	3	Chrom6/LGII	38.3	33.9-47.7	PsCam031050_18310_2958	4.3	-0.2670	8.31
Vig-Ps3.1	VF16	4	Chrom5/LGIII	165.0	150.0-191.5	PsCam005343_4052_245	3.7	0.5954	41.09
	VGH1	4	Chrom5/LGIII	170.0	168.7-178.1	PsCam005343_4052_245	9.9	0.4018	16.09
	VGH2	4	Chrom5/LGIII	188.3	164.6-195.8	PsCam005343_4052_245	5.4	0.3635	27.96
	VGHB	4	Chrom5/LGIII	185.3	163.6-195.2	PsCam005343_4052_245	4.7	0.3577	18.31
Vig-Ps3.2	VFB	4	Chrom5/LGIII	293.6	286.9-299.9	PsCam020937_11699_2576	3.8	0.5743	29.25
Vig-Ps4.1	VF15	5	Chrom4/LGIV	44.51	43.9-45.0	PsCam048119_30866_192	3.3	0.3010	24.04
	VFB	5	Chrom4/LGIV	41.2	38.1-42.5	PsCam048119_30866_192	5.2	0.4286	15.91
Vig-Ps4.2	VF16	5	Chrom4/LGIV	54.0	53.0-56.0	PsCam043430_27439_1668	10.0	0.5020	29.19
	VGH2	5	Chrom4/LGIV	51.8	46.9-53.0	PsCam001086_922_203	6.8	0.3405	20.33
	VGH3	5	Chrom4/LGIV	54.6	53.0-63.6	PsCam010470_7041_259	7.8	0.3155	26.32
	VGHB	5	Chrom4/LGIV	51.8	50.1-53.0	PsCam029227_17412_2455	6.0	0.3593	14.85
Hgt-Ps3.1	HGH2	4	Chrom5/LGIII	287.6	286.6-292.7	PsCam020937_11699_2576	5.7	-35.85	18.73
	HGH3	4	Chrom5/LGIII	287.6	286.8-293.3	PsCam020937_11699_2576	4.7	-28.88	13.43
	HGHB	4	Chrom5/LGIII	287.6	287.3-293.0	PsCam020937_11699_2576	5.5	-59.71	18.28
Hgt-Ps3.2	HGH2	4	Chrom5/LGIII	304.3	304.1-307.4	AA5	4.3	-30.90	14.55
	HGHB	4	Chrom5/LGIII	304.3	304.1-305.4	AA5	5.1	-56.51	17.11
Hgt-Ps4.1	HGH2	5	Chrom4/LGIV	13.7	10.3-14.5	PsCam000228_198_1085	3.1	25.00	10.04
	HGH3	5	Chrom4/LGIV	13.7	12.4-15.8	PsCam026907_15634_313	4.8	37.92	15.25
	HGHB	5	Chrom4/LGIV	13.7	12.9-14.5	PsCam026907_15634_313	5.9	78.84	18.64
Hgt-Ps5.1	HGH1	6	Chrom3/LGV	1.1	0.0-5.3	PsCam017782_10917_295	3.3	-16.5234	6.26
Hgt-Ps7.1	HGH1	9	Chrom7/LGVII	124.3	116.9-124.7	PsCam021891_12310_347	7.2	59.9707	17.96
	HGH2	9	Chrom7/LGVII	132.3	131.9-134.0	PsCam038582_23600_1599	6.2	39.9182	16.46
	HGH3	9	Chrom7/LGVII	132.3	132.0-134.0	PsCam038582_23600_1599	8.6	37.2430	24.89
	HGHB	9	Chrom7/LGVII	132.3	131.9-134.0	PsCam038582_23600_1599	6.2	66.1972	15.83

[α] Pea chromosomes named according to Neumann et al. (2002) and [β] Pea linkage groups named according to Tayeh et al. (2015)

Table 2.4. 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.

Epistaic-effect QTL	Trait abbrev.	Chrom	QTL 1 position	Left Marker QTL 1	Chrom	QTL 2 position	Left Marker QTL 2	R^2 (%)	Linked additive-effect QTL
E.Ae$_{MRCD1}$-Ps1	DSF15	II	10	PsCam043049_27080_440	III	182	PsCam005343_4052_245	9.7	*Ae$_{MRCD1}$-Ps2.1*, *Ae$_{MRCD1}$-Ps3.1*, *Vig-Ps2.1* and *Vig-Ps3.1*
E.Ae$_{MRCD1}$-Ps2	DSF16	III	345	PsCam004460_3351_975	III	390	PsCam029411_17551_1348	8.9	*Hgt-Ps3.2*
E.Ae$_{MRCD1}$-Ps3	DSF16	III	390	PsCam029411_17551_1348	VI	60	PsCam011542_7868_781	10.2	*Fwt-Ps4.1*
E.Ae$_{MRCD1}$-Ps4	DSGH1	Ia	35	PsCam000453_395_924	III	170	PsCam005343_4052_245	8.3	*Vig-Ps1.1*, *Ae$_{MRCD1}$-Ps3.1* and *Vig-Ps3.1*
E.Ae$_{MRCD1}$-Ps5	DSGH1	III	5	PsCam000647_565_2039	III	185	PsCam005343_4052_245	8.9	*Ae$_{MRCD1}$-Ps3.1* and *Vig-Ps3.1*
E.Ae$_{MRCD1}$-Ps6	DSGH1	II	245	PsCam001889_1542_1317	III	190	PsCam005343_4052_245	7.6	*Ae$_{MRCD1}$-Ps3.1* and *Vig-Ps3.1*
E.Ae$_{MRCD1}$-Ps7	DSGH2	IV	35	PsCam054029_35722_104	IV	55	PsCam010470_7041_259	9.1	*Ae$_{MRCD1}$-Ps4.1, Fwt-Ps4.1, Fwt-Ps4.2, Vig-Ps4.1, Vig-Ps4.2* and *Hgt-Ps4.1*
E.Ae$_{MRCD1}$-Ps8	DSGH2	III	325	AA5	IV	195	PsCam011134_7551_2658	9.0	*Hgt-Ps3.2*
E.Ae$_{MRCD1}$-Ps9	DSGH3	III	85	PsCam050501_33079_1023	III	165	PsCam005343_4052_245	12.4	*Ae$_{MRCD1}$-Ps3.1* and *Vig-Ps3.1*
E.Ae$_{MRCD1}$-Ps10	DSGH3	III	175	PsCam005343_4052_245	Vb	35	PsCam004097_3107_446	8.5	*Ae$_{MRCD1}$-Ps3.1* and *Vig-Ps3.1,*
E.Ae$_{MRCD1}$-Ps11	DSGHB	III	194	PsCam005343_4052_245	IV	30	PsCam054029_35722_104	9.0	*Ae$_{MRCD1}$-Ps3.1*, *Ae$_{MRCD1}$-Ps4.1*, *Fwt-Ps4.1, Vig-Ps3.1* and *Vig-Ps4.2,*
E.Fwt-Ps1	DFFB	III	318	AA5	III	348	PsCam042783_26826_1395	8.0	*Hgt-Ps3.2*
E.Fwt-Ps2	DFFB	III	196	PsCam005343_4052_245	VII	206	PsCam033614_19198_1651	8.6	*Ae$_{MRCD1}$-Ps3.1*, *Ae$_{MRCD1}$-Ps7.2* and *Vig-Ps3.1,*
E.Fwt-Ps3	DFGH2	Ia	130	PsCam026762_15513_1619	VI	75	PsCam042529_26584_303	8.0	*Fwt-Ps6.2*
E.Fwt-Ps4	DFGHB	III	170	PsCam005343_4052_245	III	246	PsCam042923_26960_468	8.3	*Ae$_{MRCD1}$-Ps3.1* and *Vig-Ps3.1,*

Table 2.4 continued. 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.

Epistaic-effect QTL	Trait abbrev.	Chrom	QTL 1 position	Left Marker QTL 1	Chrom	QTL 2 position	Left Marker QTL 2	R^2 (%)	Linked additive-effect QTL
E.Vig-Ps1	VF16	VI	55	PsCam011542_7868_781	VI	85	PsCam042529_26584_303	12.9	*Fwt-Ps6.2*
E.Vig-Ps2	VFB	III	176	PsCam005343_4052_245	IV	42	PsCam006741_5013_603	9.9	*Ae$_{MRDC1}$-Ps3.1, Ae$_{MRDC1}$-Ps4.1, Vig-Ps3.1, Fwt-Ps4.2* and *Vig-Ps4.2,*
E.Vig-Ps3	VGH1	Ia	25	PsCam001003_854_449	III	170	PsCam005343_4052_245	9.8	*Ae$_{MRDC1}$-Ps3.1* and *Vig-Ps3.1,*
E.Vig-Ps4	VGH1	Ib	10	PsCam011366_7739_322	III	175	PsCam005343_4052_245	7.7	*Ae$_{MRDC1}$-Ps3.1* and *Vig-Ps3.1,*
E.Vig-Ps5	VGH1	III	170	PsCam005343_4052_245	VII	50	PsCam017623_10858_46	7.6	*Ae$_{MRDC1}$-Ps3.1* and *Vig-Ps3.1,*
E.Vig-Ps6	VGH2	III	165	PsCam005343_4052_245	III	375	PsCam019069_11310_393	7.8	*Ae$_{MRDC1}$-Ps3.1* and *Vig-Ps3.1,*
E.Vig-Ps7	VGH2	II	15	PsCam043049_27080_440	III	190	PsCam005343_4052_245	7.9	*Ae$_{MRDC1}$-Ps2.1* and *Vig-Ps2.1,*
E.Vig-Ps8	VGH2	III	180	PsCam005343_4052_245	III	255	AB68	8.0	*Ae$_{MRDC1}$-Ps3.1* and *Vig-Ps3.1,*
E.Vig-Ps9	VGHB	III	164	PsCam005343_4052_245	VI	92	PsCam044306_28203_2459	8.0	*Ae$_{MRDC1}$-Ps3.1, Vig-Ps3.1* and *Fwt-Ps6.2,*
E.Hgt-Ps1	HGH3	III	340	PsCam004460_3351_975	III	345	PsCam004460_3351_975	21.4	*Hgt-Ps3.2*
E.Hgt-Ps2	HGH3	III	340	PsCam004460_3351_975	VII	105	PsCam048182_30927_2354	8.4	*Hgt-Ps3.2, Ae$_{MRDC1}$-Ps7.1, Vig-Ps7.1* and *Fwt-Ps1.1,*
E.Hgt-Ps3	HGHB	III	70	PsCam037292_22386_1574	III	78	AD270	10.4	-

Table 2.5. ANOVA: Year-station effect for root rot severity, foliar weight, vigor and plant height in field experiments conducted in Morden, MB, in 2015 and 2016 and under three greenhouse conditions.

Source of Variance	df	Mean square in field			df	Mean square in greenhouse			
		DSF	VF	FF		DSGH	VGH	FGH	HGH
Genotype (G)	134	4.5***	2.4***	32.8***	134	29.8***	5.7***	17.1***	106944***
Year-station	1	32.5***	42.1***	1462.8***	2	520***	6.1***	66.1***	363323***
Rep	2	3.5**	2.1**	5.8	7	10.1*	0.4	3.5	8175*
G*Y-S	131	1.3***	0.8***	8.5***	259	11.4***	2.0***	5.1***	7475***
Residuals	531	0.6	0.4	3.7	2707	4.09	0.9	2.3	3373

Note: Significance difference codes: 0 '***'; 0.001 '**'; 0.01 '*'; 0.05 '.'; 0.1 ' '.

Table 2.6. The heritability (h^2) of traits in each experiment and the pooled greenhouse and field data.

	Greenhouse Experiment				Field Trials		
Trait	1	2	3	BLUPs	2015	2016	BLUPs
Root rot severity	0.7	0.8	0.78	0.60	0.91	0.92	0.75
Dry foliar weight	0.67	0.72	0.78	0.73	0.91	0.94	0.78
Vigor	0.74	0.74	0.73	0.62	0.90	0.86	0.71
Height	0.91	0.92	0.93	0.94	-	-	-

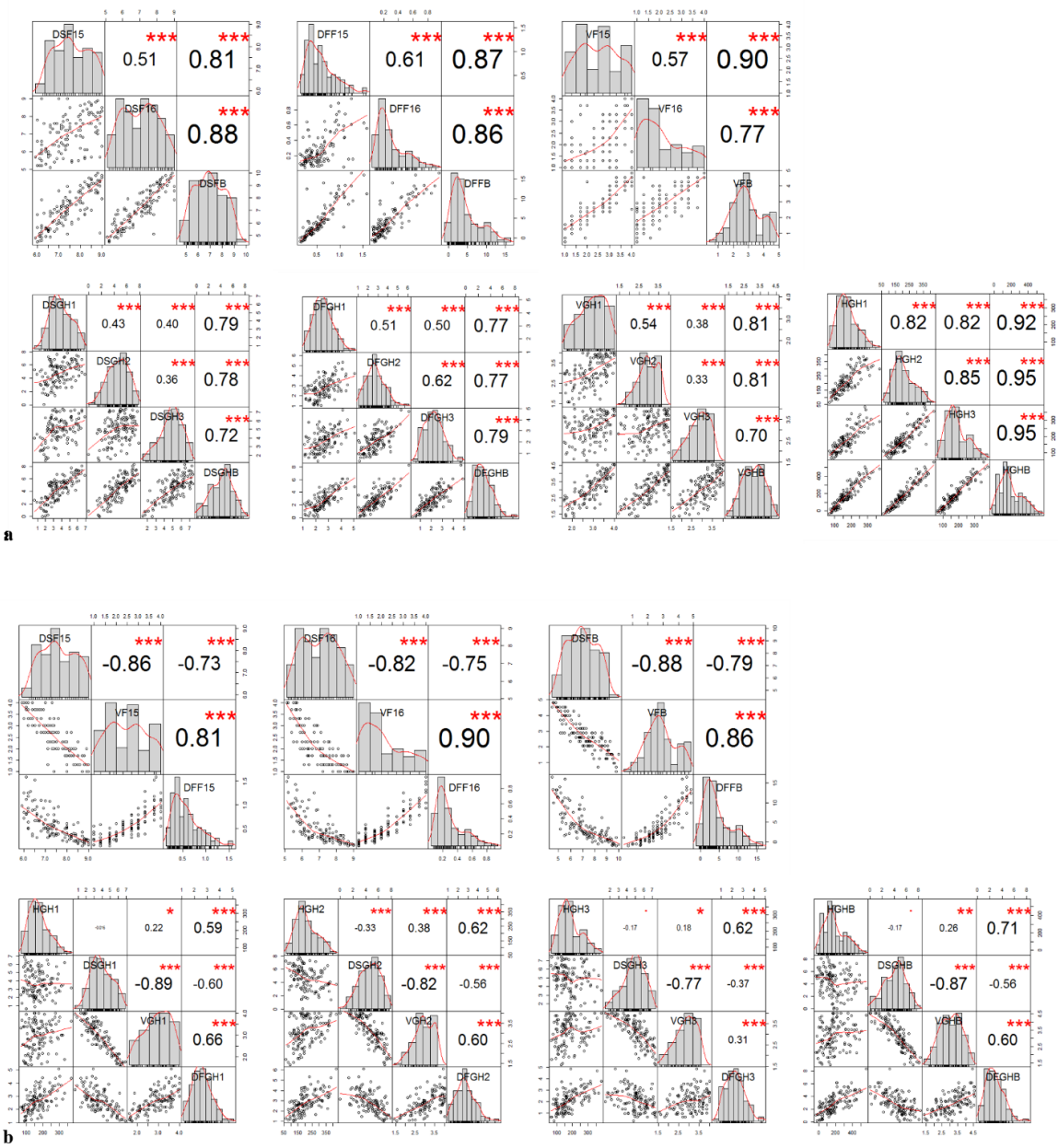


Figure 2.1. Correlation analysis of **a** all the single environment means and BLUPs of Aphanomyces root rot disease severity, pea dry foliar weight, vigor and plant height under field and greenhouse conditions, indicating the coincidence among single environments and BLUPs; and **b** relationship among all the traits in each single environment and BLUPs. The frequency distributions are shown in the bar graphs across the diagonal. The correlation coefficients and scatter plots between pairs are indicated above and below the diagonal, respectively. The significance levels are noted with asterisks, where *indicates $P < 0.05$; **indicates $P < 0.01$; and ***indicates $P < 0.001$

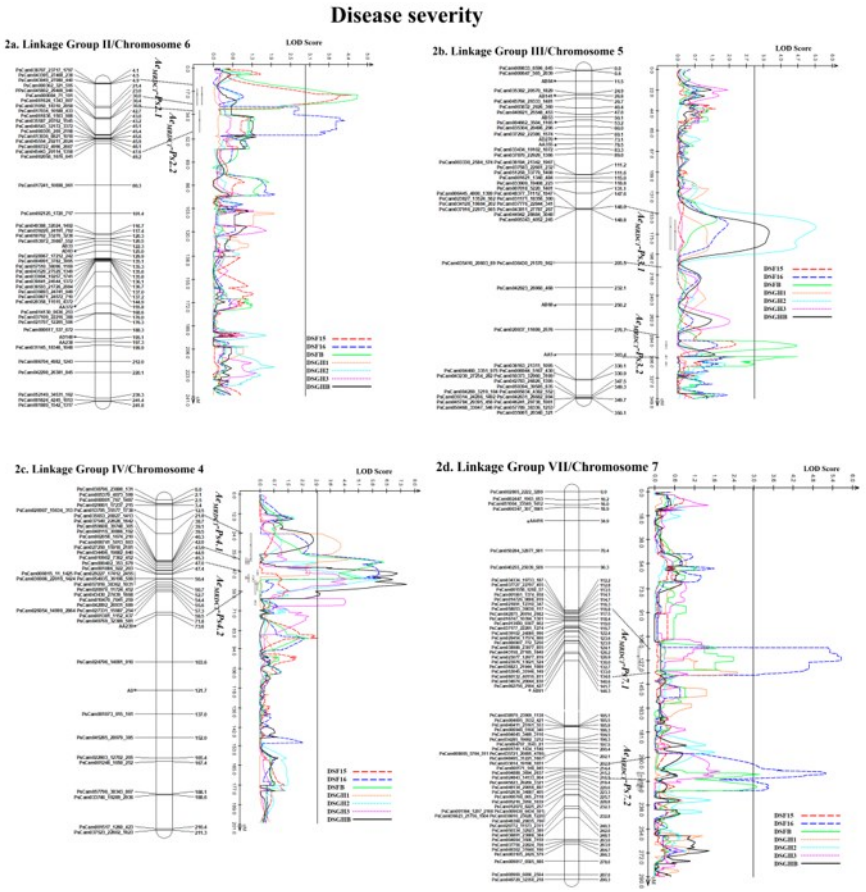


Figure 2.2. QTL likelihood profile and linkage map of pea LG II (chrom 6), III (chrom 5), IV (chrom 4) and VII (chrom 7) for partial resistance to Aphanomyces root rot in an F8 RIL of the cross 'Reward' × '002,067'. The LOD scores are indicated on the x-axis, while the genetic distances (in cM) are indicated on the y-axis. **a** Two moderate-effect QTL on LG II (chrom 6) were detected, *AeMRCD1Ps-2.1* in the 2015 field experiment and in the BLUPs of the greenhouse data, and *AeMRCD1Ps-2.2* in the 2016 field experiment. **b** The largest major effect QTL, *AeMRCD1Ps-3.1* on LG III (chrom 5), was detected in greenhouse experiment 2 and in the BLUPs of the greenhouse, while a second QTL, *AeMRCD1Ps-3.2,* was detected in the BLUPs of the field data. **c** The most stable major-effect QTL *AeMRCD1Ps-4.1* and *AeMRCD1Ps-4.2*, located in close proximity on LG IV (chrom 4), were detected in the 2016 field experiment, in the greenhouse experiments 1, 2 and 3, as well as in the BLUPs of the field and greenhouse data. **d** Two minor- to moderate-effect QTL, *AeMRCD1Ps-7.1* and *AeMRCD1Ps-7.2* on LG VII (chrom 7), were detected only in the 2016 field experiment.

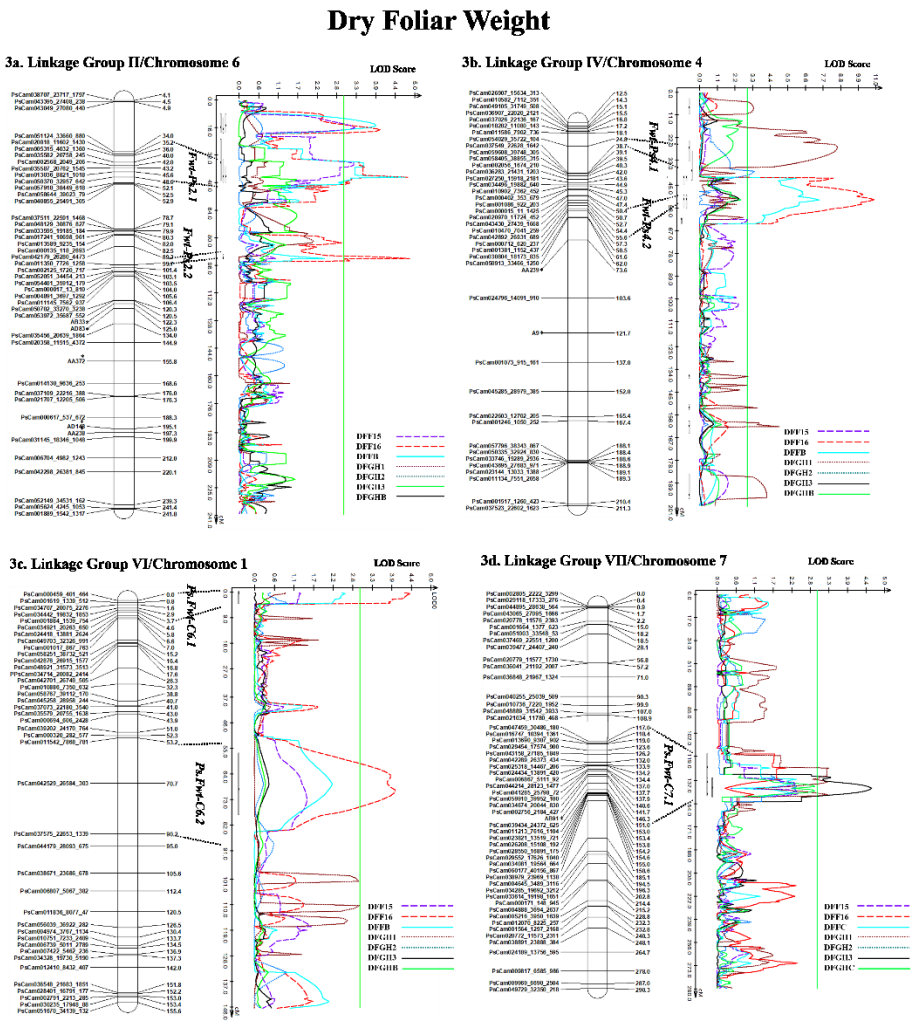


Figure 2.3. QTL likelihood profile and linkage map of pea LG II (chrom 6), IV (chrom 4), VI (chrom 1) and VII (chrom 7) for foliar weight in an F8 RIL of the cross 'Reward' × '002,067'. The LOD scores are indicated on the x-axis, while the genetic distances (in cM) are indicated on the y-axis. **a** Two minor-effect QTL on LG II (chrom 6) were detected, *Fwt-Ps2.1* in the 2016 field experiment and in the BLUPs of the field data, and *Fwt-Ps2.2* in the 2016 field experiment. **b** One moderate-effect QTL *Fwt-Ps4.1* on LG IV (chrom 4) was detected in greenhouse experiment 1, while the moderate-major-effect QTL *Fwt- Ps4.2* on the same chromosome was detected in the 2016 field experiment, in the greenhouse experiment 2 and in the BLUPs of the field data. **c** One minor-effect and one moderate-effect QTL, *Fwt-Ps6.1* and *Fwt-Ps6.2*, were detected on LG VI (chrom 1) in the 2016 field experiment. **d** One major-effect QTL, *Fwt-Ps7.1* on LG VII (chrom 7), was detected in the greenhouse experiments 1 and 3.

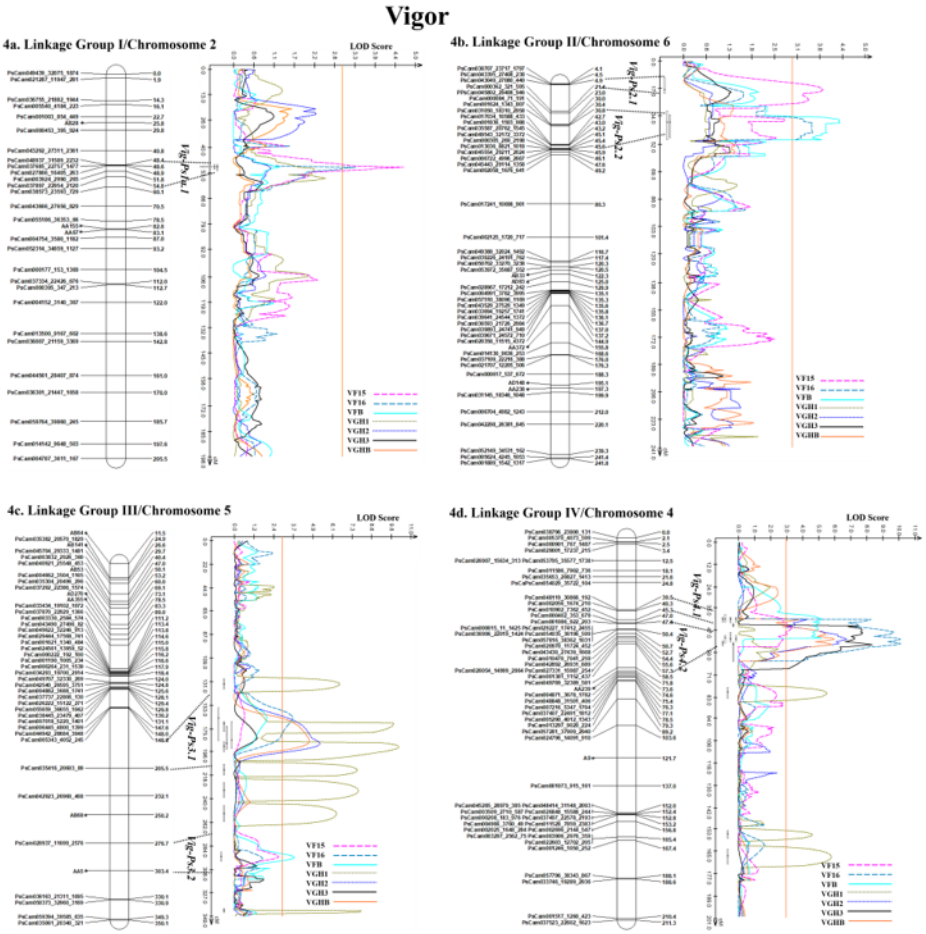


Figure 2.4 QTL likelihood profile and linkage map of pea LG I (chrom 2), II (chrom 6), III (chrom 5) and IV (chrom 4) for vigor in an F8 RIL of the cross 'Reward' × '002067'. The LOD scores are indicated on the x-axis, while the genetic distances (in cM) are indicated on the y-axis. **a** One moderate-effect QTL *Vig-Ps1.1* on LG I (chrom 2) was detected in the 2015 and 2016 field experiments. **b** Two QTL were detected on LG II (chrom 6); the moderate-effect QTL *Vig-Ps2.1* was detected in the 2015 field experiment, while the minor effect QTL *Vig-Ps2.2* was detected in the 2016 field experiment and the BLUPs of the field data. **c** Two major-effect QTL were detected on LG III (chrom 5); *Vig-Ps3.1* was detected in the 2016 field experiment and in the greenhouse experiments 1 and 2, as well as in the BLUPs of the greenhouse data, while *Vig-Ps3.2* was detected in the BLUPs of the field data. **d** The most stable major-effect QTL *Vig-Ps4.1* and *Vig-Ps4.2*, which are located in close proximity on LG IV (chrom 4), were detected in the 2015 field experiment, in the greenhouse experiments 2 and 3, as well as in the BLUPs of the field data.

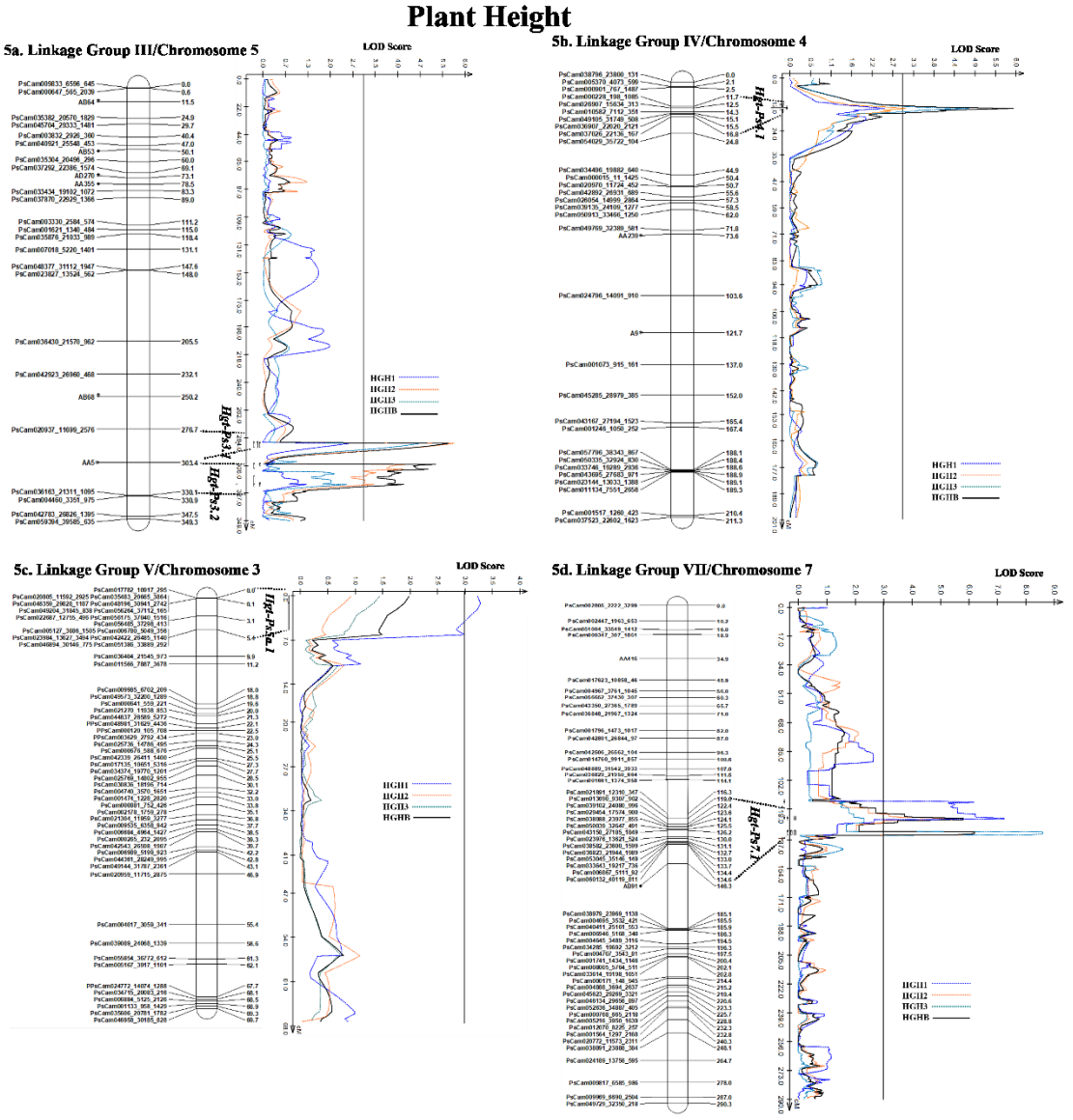


Figure 2.5. QTL likelihood profile and linkage map of pea LG III (chrom 5), IV (chrom 4), V (chrom 3) and VII (chrom 7) for height in an F8 RIL of the cross 'Reward' × '002,067'. The LOD scores are indicated on the *x*-axis, while the genetic distances (in cM) are indicated on the *y*-axis. The QTL for plant height *Hgt-Ps3.1* and *Hgt-Ps3.2* (moderate-effect QTL), *Hgt-Ps4.1* (moderate-effect QTL), *Hgt-Ps5.1* (minor-effect QTL) and *Hgt-Ps7.1* (moderate-major-effect QTL) located on LG III (chrom 5), IV (chrom 4), V (chrom 3) and VII (chrom 7) were detected consistently in the greenhouse experiments and in the BLUPs of the greenhouse data.

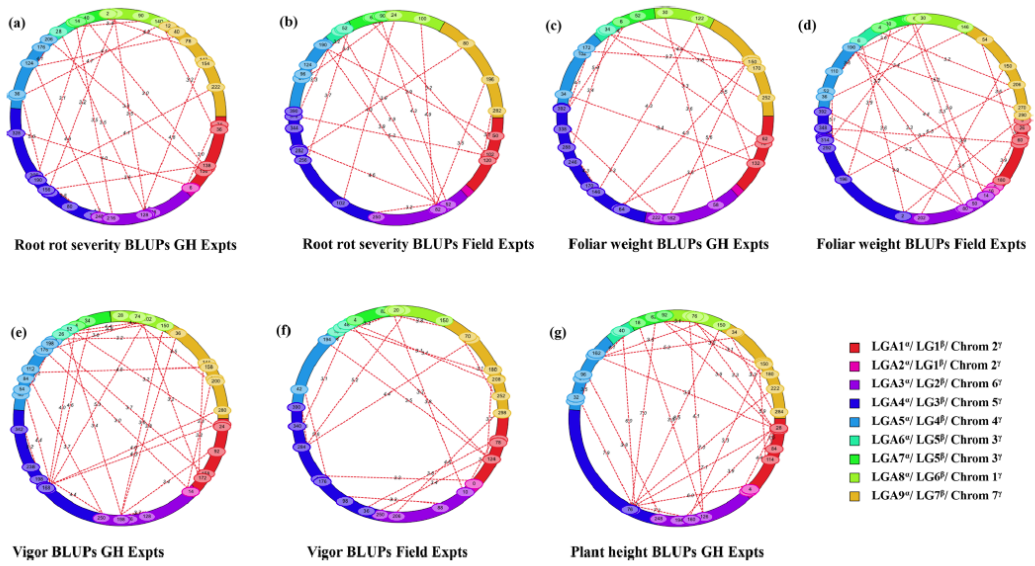


Figure 2.6. Epistatic QTL conferring tolerance to Aphanomyces root rot of pea in the pooled **a** greenhouse and **b** field data; dry foliar weight in the pooled **c** greenhouse and **d** field data; vigor in the pooled **e** greenhouse and **f** field data; and plant height in **g** the pooled greenhouse 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. $^{\alpha}$LGA-Linkage analysis according to the present study; $^{\beta}$LG-Pea linkage groups named according to Tayeh et al. (2015) and $^{\gamma}$Chrom-Pea chromosomes named according to Neumann et al. (2002)

Chapter 3 Identification of quantitative trait loci associated with partial resistance to Fusarium root rot and wilt caused by *Fusarium graminearum* in field pea

3.1 Introduction

Globally, root rot is estimated to cause yield reductions of 10 to 30% in pulse crops, but losses can be as high as 100% in crops with severe infections under ideal environmental conditions (Oyarzun 1993; Schneider et al. 2001; Schwartz et al. 2005; Cichy et al. 2007). As such, root rot is one of the most devastating diseases of field pea and other pulse crops in Canada and worldwide (Hwang and Chang 1989; Feng et al. 2010; Chatterton et al. 2015, 2019; Gossen et al. 2016; Chang et al. 2017; Safarieskandari et al. 2020; Wu et al. 2021). The causal organisms of the pea root rot complex are soil-borne fungal and fungal-like organisms that include *Fusarium* spp., *Aphanomyces euteiches*, *Pythium* spp., *Phytophthora* spp., *Rhizoctonia* spp., *Didymella* spp. (formerly *Mycosphaerella* spp.) and *Ascochyta* spp., (Xue et al. 1998; Fletcher et al. 1991; Kaiser 1992; Hwang et al. 1994; Bailey et al. 2003; Tyler et al. 2007; Díaz Ariaz 2011; Chang et al. 2005, 2013, 2014, 2017).

Given their abundance and wide host range, the vast majority of the PRRC are *Fusarium* species, although these may exhibit variable virulence towards different hosts. The various species identified in the Canadian prairies include *F. Solani, F. avenaceum, F. oxysporum, F. graminearum F. culmorum, F. graminearum, F. acuminatum, F. redolens F. sambucinum var. coeruleum, F. equiseti, F. poae, F. sporotrichioides, and F. tabacinum* (Kraft and Pfleger 2001; Fernandez 2007; Fernandez et al. 2008; Feng et al. 2010; Chittem et al. 2015; Wu et al. 2017; Chang et al. 2018; Zitnick-Anderson et al. 2018). Among these, *F. avenaceum*, *F. solani,* and *F. oxysporum* were reported to be the primary species causing significant Fusarium root rot in the major field pea cultivation regions in Canada and worldwide (Kraft et al. 1981; Kraft and Pfleger 2001; Wille et al. 2020).

The *Fusarium graminearum* species complex includes the major pathogens causing Fusarium head blight (FHB) of wheat, barley, oats and other small grain cereals (O'Donnell 2008). On cereal

hosts, FGSC produces various mycotoxins known as trichothecenes (e.g., deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEN), and fumonisin B1 (FB1)), which are detrimental to human and animal health when ingested (van der Lee et al. 2015). While *F. graminearum* mainly affects cereals, this pathogen has been isolated from field pea in Canada, the USA and Lithuania (Feng et al. 2010; Chittem et al. 2015; Rasiukevičiūtė et al. 2019). Rasiukevičiūtė et al. (2019) reported that field pea was the non-cereal crop most susceptible to *F. graminearum* compared with faba bean, fodder beet, oilseed rape, potato and sugar beet.

At present, there are no sources of complete resistance to PRRC in field pea. Furthermore, higher global temperatures and excessive soil moisture associated with climate change have led to the increased incidence and severity of many plant diseases (Chakraborty et al. 2000; Dorrance et al. 2003; Gautam et al. 2013; Elad and Pertot 2014). While tillage was reported to be beneficial to the soil environment, it did not suppress the development of Fusarium root rot in field pea (Bailey et al. 1992). Seedling data and depth were reported to affect Fusarium root rot in lentil (Hwang et al. 2000), but not in field pea (Chang et al. 2013). Longer crop rotations of more than 4 years are recommended for the management of root rot, but these are not always practical (Hwang et al. 1989; Bainard et al. 2017). Fungicidal seed treatments were reported to increase emergence and reduce root rot severity in the early growth stages of pea (Xue et al. 2000; Wu et al. 2019), with Apron Maxx (fludiozonil and metalyxyl-M and S-isomaer), prothioconazole, fluopyram and penthiopyrad suppressing Fusarium root rot in greenhouse and field experiments (Avenot et al. 2010; Chang et al. 2013). However, some fungicides can also affect Rhizobia, leading to reductions in nodulation and nitrogen fixation (Chang et al. 2013), and their use is not necessarily environmentally friendly.

Genetic resistance offers the most promising way to control Fusarium root rot and wilt in pea. However, there is no complete resistance Fusarium root rot in field pea, and only a few studies have reported QTL associated with partial resistance to this disease (Feng et al. 2011; Mc Phee et al. 2012; Coyne et al. 2015; 2019). Coyne et al. (2015; 2019) identified a major QTL for partial resistance to *F. solani*, *Fsp-Ps2.1*, to be on LGII (Chromosome 6), while four minor QTL were found on LGIII, IV, VI and VII (chromosomes 5, 4, 1 and 7, respectively). This QTL explained

44.4% to 53.4% of the total variance for resistance (Coyne et al. 2019). Mc Phee et al. (2012) detected one major QTL on LGIV (chromosome 4) and two minor QTL on LGIII (chromosome 5) to be associated with partial resistance to *F. oxysporum* race 2. A major QTL, *Fnw4.1*, explained 68% to 80% of the phenotypic variance. Feng et al. (2011) identified one QTL controlling resistance to *F. avenaceum* on LGVII (Chromosome 7) in a rough map generated with 14 SSRs. The QTL identified in most of these studies had very large confidence intervals due to the limited number of markers used. The low marker density makes it difficult to apply the identified markers in pea breeding.

On the Canadian prairies, cereals are grown in tight rotations with canola, while the cultivation of field pea and other pulses is increasing (Bekkering 2013; Gill 2018). Boom-and-bust type cycles of root rot diseases were highly correlated with crop rotation practices (Govaerts et al. 2007; Su et al. 2021). Therefore, the order of cultivation of crops in a rotation is important. The increased incidence and severity of fusarium root rot (FRR) in field pea makes the study of the genetic resistance to different *Fusarium spp.* an important research objective.

Therefore, the objectives of this study were to (1) evaluate the partially resistant pea cultivar '00-2067' for resistance to different Fusarium species recovered from surveys for root rot in Alberta, Canada; (2) map the QTL associated with partial resistance to Fusarium root rot using a segregating RIL pea population and the most virulent of the Fusarium isolates; and (3) determine the stability of the QTL accounting for disease severity, vigor and plant height.

3.2 Materials and Methods

3.2.1 Plant Materials

One-hundred thirty-five recombinant inbred lines (RIL) used for mapping the QTL associated with partial resistance to Aphanomyces root rot by Wu et al. (2021) were used in this study. In brief, the Aphanomyces root rot resistant pea parent '00-2067' (Conner et al. 2013; Wu et al. 2021) was used in genetic crosses with the susceptible parent 'Reward' (Bing et al. 2006) to produce F_1

plants, which were then used to develop an F_8 RIL population by the single-seed descent (SSD) method (Brim 1966).

3.2.2 Fusarium isolates

Five fusarium single-spore isolates (SSI), S4C (*F. solani*), F4A (*F. avenaceum*), F037 (*F. acuminatum*), F039 (*F. proliferatum*) and FG2 (*F. graminearum*), representing the *Fusarium* species most frequently recovered from symptomatic pea plants in root rot surveys in Alberta, were used to screen the parental cultivars '00-2067' and 'Reward'. Briefly, to obtain the SSI, surface-sterilized pieces of root tissue with disease lesions were placed on potato dextrose agar (PDA) and incubated at 25°C for 2 to 3 days and then transferred to peptone-pentachloronitrobenzene (PCNB) medium for further selection. Mycelial tips of the fungal isolates were cut from selected colonies under a stereomicroscope (Zeiss Axio Scope A1, Carl Zeiss Canada Ltd., Canada), and the water agar (WA) procedure was used to obtain SSI (Zitnick-Anderson et al. 2020). The SSI were confirmed to be *Fusarium* species based on their morphology and evaluation with species-specific PCR primers, and their virulence was confirmed using Koch's postulates (Feng et al. 2010; Chen et al. 2014; Zhou et al. 2014; Wu et al. 2017; Chang et al. 2018; Zitnick-Anderson et al. 2018).

3.2.3 Inoculum production

Conidial suspensions of the five isolates were generated following Son et al. (2013). Pure cultures of each *Fusarium* spp. were grown in Petri dishes on PDA under darkness at room temperature for 4-6 weeks. Sterile distilled water was added to each Petri dish and the surface of each colony was gently scraped with a sterile inoculation needle to dislodge the spores (and mycelia fragments), with the resulting suspension decanted into 250 mL Erlenmeyer flasks containing 100 mL autoclaved CMC medium (1.5% carboxymethyl cellulose, 0.1% yeast extract, 0.05% $MgSO_4 \cdot 7H_2O$, 0.1% NH_4NO_3, 0.1% KH_2PO_4, 100 mL H_2O). The flasks were covered in aluminum foil to block light and incubated on a rotary shaker at room temperature for 2 weeks. The suspension was centrifuged to collect conidia. The concentration of conidia was estimated using a

haemocytometer and diluted to a final concentration of 2×10^6 oospores mL^{-1} with sterile deionized water.

3.2.4 Screening of RIL parents with five *Fusarium* species

Plastic cups (9 cm in diameter and 10.5 cm depth) were filled with a sterilized potting mixture (Cell-TechTM, Monsanto, Winnipeg, MB). In the greenhouse tests with each isolate (S4C, F4A, F037, F039 and FG2), the roots of seven 5-day-old seedlings of the partially resistant parent '00-2067' and the susceptible parent 'Reward' were immersed in the conidial suspension for 15 mins and transplanted into the soilless mixture in a cup. An aliquot (1 ml) of conidial suspension was pipetted onto the roots before they were covered with the potting mix. The plants were kept in a greenhouse at 20-25°C/15-18°C day/night and a 16 h photoperiod with daily watering to maintain the potting mix at saturation conditions conducive for FRR development. Each experiment was repeated twice. After 3 weeks, disease severity was estimated on a scale of 0 to 4, where: 0 = completely healthy; 1 = brown or black spots on main root; 2 = lesions cover the main root, but the rootlet is still healthy; 3 = lesions spread to the entire root system; and 4 = root totally dead.

3.2.5 Disease assessment of RIL population under controlled conditions

The most virulent of the *Fusarium* isolates was used as inoculum to screen 135 RIL population and the parents under greenhouse conditions. The inoculation and maintenance of the plants were as described above. The pots were arranged in a randomized complete block design with four replicates. The greenhouse experiment was repeated four times. After 3 weeks, plant height was measured from the base of the stem to top leaf with units of centimeter. Plant vigor was evaluated as a measure of the wilting severity on a scale of 0 to 4 (4 = plant completely healthy; 3 = thin stem and short height; 2 = brown lesions on stem and yellowing of leaf tips; 1 = wilting on stem and leaves; 0 = plant completely dead). The plants were then carefully uprooted, washed under standing water, and assessed for disease severity as described above.

3.2.6 Statistical analysis of phenotypic data

Analysis of variance (ANOVA) was conducted using R software (R core team 2019) for disease severity, vigor and plant height in four greenhouse environments. The mean and least square mean of all traits were calculated for single environments and total data in R (package 'lsmenas'). To estimate random effects, the best linear unbiased predictors (BLUPs) and heritability were also calculated using the package 'Phenotype' in R (R core team 2019). Correlation analysis was conducted within each trait (all variables including means for single environments, LSM and BLUPs for total data) and among traits (including disease severity, vigor and plant height) using the package 'PerformanceAnalytics' in R, displaying the correlation coefficient, frequency distribution and dot plot. The Shapiro-Wilk test was conducted to examine the normality for each variable.

3.2.7 Genotyping with SNP and SSR markers

The 13.2K SNP markers and 222 SSR markers, the parents and the RIL population genotyped by Wu et al. (2021) were used in this study. In brief, SNP genotyping was carried out at TraitGenetics GmbH, Gatersleben, Germany, using sequences developed by Tayeh et al. (2015). The SSR markers were obtained from Loridon et al. (2005). In the case of the SSR markers, the PCR assays, thermal cycling conditions and genotyping using an ABI PRISM 3730xl DNA analyzer (Applied Biosystems, Foster city, CA) were as described by Wu et al. (2021). Filtering of the SNP and SSR was carried out to retain highly polymorphic markers and RIL individuals with >95% genotyping data, as well as markers that exhibited the expected 1:1 segregation ratio.

3.2.8 Linkage map construction

Linkage analysis was carried out using the filtered SNP and SSR markers following Wu et al. (2021). This involved the generation of a draft linkage map using the minimum spanning tree map (MSTMap) (Wu et al. 2008) and then refined by MAPMAKER/EXP 3.0 (Lincoln et al. 1992). The Kosambi map function (Kosambi 1944) was used to calculate the genetic distances (in cM) between the markers. The map construction was carried out with MapChart v. 2.32 (Voorrips 2002) using

the Kosambi map function, of which the linkage groups were assigned to chromosomes based on the consensus SNP map of pea developed by Tayeh et al. (2015).

3.2.9 QTL Analysis

Additive-effect QTL analysis was first carried out using the genotype and phenotype data (disease severity, vigor and plant height) from the RILs inoculated with *F. graminearum* (FG2). This was then repeated for the RILs inoculated with *F. avenaceum* (F4A). The analysis was carried out using means for the four single greenhouse experiments, LSM and BLUPs of the total data by Composite Interval Mapping using WinQTL Cartographer v2.5. The program was set at 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. 2012). The LOD score threshold was set at 3.0 for QTL detection. The 95% confidence interval for each trait was defined by the consensus region bordered by the four environments.

The QTL names were defined according to QTL detection studies by Coyne et al. (2015, 2019), where the name of the *Fusarium* isolate was indicated followed by "*Ps*" = *Pisum sativum*, the first number = pea linkage group (Tayeh et al. 2015), and the second number = the serial number of the QTL on the linkage group. For example, '*Fg-Ps4.1*' represents the QTL for disease severity caused by *F. graminearum* located on linkage group IV of the pea genome. The chromosome and pseudomolecules were named in accordance with Neumann et al. (2002) and Kreplak et al. (2019), respectively. A similar nomenclature was adopted for vigor (*Vig-Ps2.1*) and plant height (*Hgt-Ps2.1*).

A QTL identified in at least two of the four environments was classified as stable. The percentage of variation (R^2) was determined for each QTL. Furthermore, QTL with R^2 >10%, 5-10% and <5% were arbitrarily classified as major, moderate or minor-effect QTL, respectively. The origins of favorable alleles for individual traits were assigned to different parents following Wu et al. (2021). Pairwise epistatic interactions were estimated with IciMapping V.4.1 using the ICIM-EPI method (Meng et al. 2015). The significance threshold for major, moderate and minor were arbitrarily set at R^2 >15%, 7.5-15% and <7.5%, respectively. Epistatic-effect QTL were

named with the prefix "*E*" followed by the QTL name and a serial number (e.g. *E.FG-Ps1*, *E.Vig-Ps1* and *E.Hgt-Ps1*).

3.3 Results

3.3.1 Preliminary root rot assessment in parents against five *Fusarium* spp.

Between the parental cultivars, '00-2067' developed lower root rot severity than 'Reward' in response to each of the five isolates (Table 3.5), confirming that '00-2067' was tolerant while 'Reward' was susceptible. There was a significant difference ($p < 0.001$) between the mean root rot values of the tolerant parent '00-2067' and the susceptible parent 'Reward' following inoculation with *F. graminearum* isolate FG2 and *F. avenaceum* isolate F4A, while no significant differences were detected following inoculation with the *F. solani*, *F. acuminatum* and *F. proliferatum* isolates S4C, F037 and F039, respectively (Table 3.5). Therefore, FG2 and F4A were selected to screen the 135 F_8 RIL population for QTL identification associated with resistance to FRR.

3.3.2 ANOVA for disease severity, vigor and plant height

The mean root rot severity, vigor and plant height of the RIL population inoculated with FG2 and F4A are presented in Tables 3.1 and 3.2. ANOVA indicated that the genotypic effect of disease severity, vigor and plant height was significant ($P<0.001$) (Tables 3.6a and 3.6b). This suggested that a high proportion of genetic variance was transmitted from parental cultivars to the progenies. Heritability values of 92% and 86% for disease severity and vigor were obtained for plants inoculated with FG2 and F4A, respectively, while heritability values for plant height ranged from 79% to 91% **(**Tables 3.3a and 3.3b**)**. The G×E interactions were significant for disease severity, vigor and plant height for F4A but not for FG2, while differences among the four greenhouse experiments were significant for both FG2 and F4A ($P<0.001$) (Tables 3.6a and 3.6b).

3.3.3 Root rot, vigor and plant height of parents and the RIL population inoculated with FG2

Estimated disease severity values (± SE) on the parental cultivar '00-2067' inoculated with FG2 were 1.5 ± 0.7, 1.3 ± 0.6, 2 ± 1.2, 1.5 ± 0.7 for the four greenhouse experiments, 1.6 ± 0.8 for LSM and 1.2 for the BLUPs. This was comparable with the estimated mean of 1.1 ± 0.4 obtained in the preliminary screening of the parents (Table 3.1 and 3.5). On the other hand, the estimated disease severity values (± SE) for 'Reward' were 3.3 ± 0.5, 3.3 ± 0.5, 3.5 ± 0.6 and 3.0 ± 0.0 for the four greenhouse experiments, 3.3 ± 0.5 for LSM and 4.1 for the BLUPs; these values were also comparable to the estimated mean of 3.3 ± 0.4 obtained in the preliminary screening (Table 3.1 and 3.5). A t-test indicated a significant difference between the parents for disease severity in all four experiments. Frequency distribution (Figure 3.1) indicated that the disease severity data of the RILs in the four experiments were continuous, but only DSGH3 and DSGHC followed a normal distribution based on the Shapiro-Wilk test (Table 3.1). High correlation coefficients, ranging from 68% to 99%, were found for disease severity among the single experiments, pooled and BLUPS (Figure 3.1). The differences in vigor between the parents inoculated with FG2 were significant, except for VGH4. The parental cultivar, '00-2067' had estimated means (± SE) of 4.0 ± 0.0, 4.0 ± 0.0, 3.0 ± 1.2, 3.5 ± 0.7 for the four greenhouse experiments and 3.6 ± 0.8 for the pooled data. In the case of 'Reward', the estimated means (± SE) were 2.0 ± 0.8, 2.5 ± 0.6, 1.5 ± 0.6, 2.7 ± 0.6 for the four greenhouse experiments and 2.2 ± 0.7 for the pooled data. The BLUPs for parental cultivars, '00-2067' and 'Reward' were 4.2 and 1.6, respectively (Table 3.1). The Shapiro-Wilk test indicated that the RIL population vigor data for the four greenhouse experiments did not follow a normal distribution, except for VGHC (Figure 3.1). A significant correlation ($0.34 < r < 0.96$, $P<0.001$) existed among the single experiments, pooled and BLUPS for vigor (Figure 3.1).The plant height of '00-2067' plants inoculated with FG2 was relatively greater than plants of 'Reward' for the means in the single environments, LSM and BLUPs, although the differences were not significant based on a t-test. The estimated means in single conditions, LSM and BLUP for plant height (± SE) of '00-2067' were 234.5 ± 54.6, 157.3 ± 50.6, 155.5 ± 59.5, 159.7 ± 6.7, 176.7 ± 56.6 and 158.9, respectively. For 'Reward', the plant heights were 177.5 ± 36.1, 120.7 ± 31.5, 129.5 ± 26.0, 178.5

± 34.6, 151.5 ± 36.9 and 100.8, respectively. The frequency distribution of plant height of the RIL population for all six variables was not normal and slightly skewed (Table 3.1 and Figure 3.1). A high correlation ($0.42 < r < 0.95$, $P < 0.001$) was found for plant height among the single experiments, pooled and BLUPS data (Figure 3.1).

Collectively, the correlation analysis among traits indicated that root rot caused by FG2 was negatively correlated with vigor and plant height. High correlation coefficients were detected between disease severity and vigor in all conditions ($-0.65 < r < -0.90$, $P < 0.001$), indicating the adverse effect of FG2 on root and aboveground growth. Plant height showed low to moderate correlation with disease severity ($-0.22 < r < -0.35$, $P < 0.05$) and vigor ($0.19 < r < 0.38$, $P < 0.05$).

3.3.4 Root rot, vigor and plant height of parents and the RIL population inoculated with F4A

The estimated means (± SE) of disease severity for '00-2067' were 1.0 ± 0.0, 1.0 ± 0.8, 1.3 ± 0.5 1.0 ± 0.0, 1.1 ± 0.4 and 1.0, while for 'Reward' they were 3.3 ± 0.5, 3.3 ± 0.5, 3.5 ±0 .6, 3.0 ± 0.0, 3.3 ± 0.4 and 3.0 for DSGH1, DSGH2, DSGH3, DSGH4, LSM of pooled data and BLUPS, respectively (Table 3.2). These values were comparable to the estimated means (± SE) of 1.8 ± 0.5 and 2.8 ± 0.2 for disease severity obtained in the preliminary screening of the parents (Table 3.2 and 3.5). T-tests indicated significant differences between estimated means of the parental cultivars, '00-2067' and 'Reward' inoculated with F4A. The Shapiro-Wilk test indicated that only the root rot data of the RIL population for DSGH4 and DSGHPooled followed a normal distribution (Table 3.2), although the data for the four greenhouse experiments were continuous (Figure 3.2). The correlation coefficient between the experiments ranged from 0.44 to 0.93 ($P < 0.001$ (Figure 3.2). Based on the t-tests, the parental cultivar '00-2067' inoculated with F4A had a significantly greater vigor than 'Reward'. The estimated vigor values (± SE) for '00-2067' were 4.0 ± 0.0, 3.7 ± 0.5, 3.5 ± 0.6 and 4 ± 0 for the four individual greenhouse experiments, 3.8 ± 0.5 for LSM for the pooled data and 4.0 for BULPs of the pooled greenhouse experiments (Table 3.2). The estimated vigor values (± SE) for 'Reward' were 1.7 ± 0.5, 2.0 ± 1.4, 1.2 ± 1.5 and 2.5 ± 0.6 for the individual greenhouse experiments, 1.9 ± 1.1 for LSM and 1.9 for the BULPs of the pooled greenhouse experiments. All vigor variables for the RIL population were continuous with slight left skewness

(-0.4 ~ -0.9) (Figure 3.2). Additionally, the data did not follow a normal distribution based on the Shapiro-Wilk test (Table 3.2). The correlation coefficient between the experiments ranged from 0.54 to 0.98 ($P < 0.001$) (Figure 3.2).

In contrast to vigor, the difference in plant height of the parental cultivars inoculated with F4A was not significant based on the t-test. The estimated plant height for '00-2067' for the individual experiments, LSM and BLUP was 210.8 ± 128.2, 174.5 ± 104.8, 159.5 ± 13.5, 210.0 ± 53.1, 188.7 ± 82.8 and 208.0, respectively. The estimated plant height for 'Reward' was 118.3 ± 100.2, 193.5 ± 104.5, 125.0 ± 98.9 and 194.0 ± 38.4 for the individual experiments, 157.7 ± 88.5 for LSM and 133.1 for the BLUP. The frequency distribution for the RIL population was continuous and slightly skewed to the right. In addition, HGH2, HGH3, HGHPooled and HGHBLUPS followed a normal distribution (Table 3.2 and Figure 3.2). Plant height variables were also significantly correlated ($0.28 < r < 0.97$, $P < 0.01$) (Figure 3.2).

The correlation among the traits for plants inoculated with F4A was similar to that of plants inoculated with FG2. Disease severity was highly correlated with vigor ($-0.88 < r < -0.95$, $P < 0.001$) and with plant height ($-0.48 < r < -0.63$, $P < 0.001$). Plant height was positively correlated with vigor ($0.57 < r < 0.62$, $P < 0.001$).

3.3.5 Genetic map construction and QTL analysis

Linkage grouping, the distribution of markers, map length and marker density of 2999 (2978 SNP + 21 SSR) retained markers on the seven chromosomes (Neumann et al. 2002), linkage groups (Tayeh et al. (2015) and pseudomolecules of pea (Kreplak et al. 2019) is described in Wu et al. (2021). The genetic map spanned 1704.1 cM and contained an average marker density of 1.8 markers/cM (Wu et al. 2021). The QTL analysis was conducted with 1422 unique markers, which represented 10.5% of the markers used for genotyping (Wu et al. 2021).

3.3.6 Additive-effect QTL analysis

No significant QTL (LOD <3.0) for disease severity, vigor and plant height were detected for the RILs inoculated with *F. avenaceum* isolate F4A. As such, no QTL likelihood profiles are shown.

In the case of RILS inoculated with *F. graminearum* isolate FG2, a total of 11 QTL were detected for the three parameters and six variables (i.e. GH1, GH2, GH3, GH4, LSM and BLUPs) by the CIM using WinQTL Cartographer v2.5 (Wang et al. 2012) (Table 3.3). Five of the 11 QTL were identified for disease severity, whereas three QTL each were detected for vigor and plant height. The QTL had LOD scores ranging from 3.0 to 14.4 and the percentage of phenotypic variation (R^2) values ranging from 4.05% to 36.35% (Table 3.3). Based on the R^2 values, two, six and three of the QTL were considered major, moderate or minor-effect, respectively. Six of the 11 QTL were identified in two or more environments and hence could be considered stable, while the remaining five QTL were detected in single experiments and hence could be considered unstable.

The most stable QTL for partial resistance to *F. graminearum* isolate FG2, *Fg-Ps4.1* and *Fg-Ps4.2*, were located in the middle of Chrom4/LGIV at positions 59.3-74.4 cM and 74.0-85.2 cM, respectively. The 15.1 cM and 11.2 cM genomic regions delimiting these two QTL were flanked by the SNP markers PsCam048871_31524_450 and PsCam001381_1152_437 and the SSR marker AA239 and SNP marker PsCam057281_37909_2940, respectively. Both *Fg-Ps4.1* and *Fg-Ps4.2* exhibited a moderate effect, with the percentage variance ranging from 9.1% to 15.4% (Table 3.3). Two other moderate-effect but unstable QTLs, *Fg-Ps3.1* (located on the bottom segment (307.9-316.5 cM) of Chrom5/LGIII and with flanking markers of AA5 and PsCam036163_21311_1095) and *Fg-Ps3.2* (located distal to *Fg-Ps3.1* and with flanking markers PsCam036163_21311_1095 and PsCam042783_26826_1395) explained 9.62% to 9.88% of the total variance. Another unstable QTL, *Fg-Ps5.1* (detected on the top part (0.9-9.2 cM) of Chrom3/LGV and flanked by the SNP markers PsCam059449_39630_321 and PsCam011153_7569_125), explained 14.2% of the total variance in greenhouse experiment 1. Four of the QTL for disease severity (with the exception of *Fg-Ps5.1*) had a negative additive effect, indicating that genomic regions for resistance in *Fg-Ps4.1, Fg-Ps4.2, Fg-Ps3.1* and *Fg-Ps3.2* originated from '00-2067', while *Fg-ps5.1* derived its resistance from 'Reward' (Table 3.3).

The stability of the QTL for vigor was in the order *Vig-Ps4.1* on Chrom4/LGIV (GH1, GH2 and GH4, R^2 = 9.19 to 13.5%) > *Vig-Ps3.2* (GH2 and GH3, R^2 = 9.53% to 12.13%) > *Vig-Ps3.1*

(GH4, R^2 = 4.05%) both on Chrom5/LGIII (Table 3.3). The QTL *Vig-Ps4.1* was located on Chrom4/LGIV from 58.0 cM to 73.2 cM between the SNP marker PsCam000712_620_237 and the SSR marker AA239. *Vig-Ps3.2*, which was located 307.8-316.5 cM on the bottom of Chrom5/LGIII, was flanked by the SSR marker AA5 and the SNP marker PsCam036163_21311_1095; *Vig-Ps3.1*, located on the top segment (67.1 to 70.5 cM) of the same chromosome or linkage group, was flanked by the SNP marker PsCam013763_9362_423 and the SSR marker AD270. The two stable QTL, *Vig-Ps4.1* and *Vig-Ps3.2*, had a positive additive effect, indicating that the alleles for vigor originated from '00-2067'. In contrast, *Vig-Ps3.1* has a negative additive effect indicating that the alleles originated from 'Reward' (Table 3.3).

In the case of plant height, the most stable QTL, *Hgt-Ps3.1,* was detected in three of the four experiments (GH1, GH2 and GH4; R^2 = 9.94% to 36.35%). This QTL was located on the bottom segment of Chrom5/LGIII (Table 2.3) and was flanked by the SNP marker PsCam020937_11699_2576 and the SSR marker AA5. The second most stable QTL, *Hgt-Ps7.2*, was detected across two (GH2 and GH4) of four greenhouse experiments (R^2 = 7.04 to 20.04%). This QTL was located 142.3 cM to 168.0 cM on Chrom7/LGVII and was flanked by the SNP markers PsCam002756_2184_427 and PsCam045262_28962_162 (Table 3.3). *Hgt-Ps7.1*, which was flanked by the SNP markers PsCam035831_20992_561 and PsCam021891_12310_347 (81.2-115.3 cM), was detected in only one environment (GH1) on the same chromosome (R^2=13.63 to 14.06%). The additive effect was negative for *Hgt-Ps3.1*, but positive for *Hgt-Ps7.1* and *Hgt-Ps7.2* (Table 3.3). This suggested that the QTL for height on Chrom5/LGIII was derived from 'Reward', while the QTL on Chrom7/LGVII originated from '00-2067'.

3.3.7 Epistatic QTL analyses

Two hundred eight putative digenic epistatic pairs were identified using all variables for disease severity, vigor and plant height. These comprised 65 (12-24) for disease severity, 57 (10-21) for vigor and 86 (15-28) for plant height. The 208 putative digenic interactions consisted of one major epistatic effect (PVE ≥ 15%), 13 moderate epistatic effect (7.5% ≤ PVE ≤ 15%) and 194 minor epistatic effect (PVE ≤ 7.5%). BLUPs for disease severity, vigor and plant height detected

20, 18 and 21 putative digenic interactions, respectively. Within the 59 digenic interactions, epistatic analysis identified one major QTL pair, three moderated QTL pairs and 55 minor QTL pairs (Table 3.4). In contrast, LSM of the pooled data detected 23 digenic interactions for disease severity, 14 for vigor and 19 for plant height. The total 56 pairs included seven moderate epistatic effect QTL and 49 minor effect QTL.

Twenty-five digenic epistatic interactions with major and moderate effects were identified by 33 flanking markers, of which 10 epistatic-effect QTL with 14 flanking markers were linked to three additive-effect QTL (*Fg-Ps3.1, Fg-Ps3.2* and *Vig-Ps3.1*). The remaining 15 epistatic QTL were not related to any of the additive-effect QTL (Table 3.4). Eight of the 10 epistatic-effect QTL were linked to *Fg-Ps3.2*, including the most significant QTL pairs, *E.Hgt-Ps1* (R^2 = 31.2%), followed by *E.Hgt-Ps7* (R^2 = 19.1%) and *E.Hgt-Ps4* (R^2 = 13.5%). The fourth was *E.Hgt-Ps3* (R^2 = 13.5%), which was linked to *Fg-Ps3.2* and *Vig-Ps3.1*. Only *E.Fg-Ps7* and *E.Vig-Ps7* were linked to Fg-Ps3.1, showing moderate epistatic effect (R^2 = 9.5% and R^2 = 12.6%, respectively).

3.4 Discussion

Commercial farming in Canada is characterized by short rotations of cereal crops with canola and to a limited extent pulse crops. Disease surveys in Canada have identified *Fusarium* species as the most frequently isolated fungi from all crops surveyed for root rot severity (K.F. Chang, unpublished data). *Fusarium poae* was predominant in FHB infected kernels, followed by *F. graminearum*; other *Fusarium* species were less common in infected kernels (Banik et al. 2019; Xue et al. 2019; Ziesman et al. 2019). The predominant *Fusarium* spp. isolated from the infected roots of field pea were *F. avenaceum*, *F. solani* and *F. oxysporum* (Kraft et al. 1981; Kraft and Pfleger 2001; Feng et al. 2010; Chittem et al. 2015; Rasiukevičiūtė et al. 2019). *Fusarium* species, especially *F. acuminatum*, have been reported to cause root rot of canola (Li et al. 2007; Chen et al. 2014).

Increasingly, *F. graminearum* has become a major problem across cereal growing regions worldwide. For example, in Manitoba, Canada, from 1937 to 1942, *F. graminearum* was present in <0.5% of 1448, 262, 865 and 519 samples, respectively, of wheat, durum, barley and oats tested,

compared with 16.4% to 39.9% for *F. poae* and 13.5% to 29.5% for *F. acuminatum* (Gordon 1944). In contrast, in Saskatchewan, Canada, from 2014 to 2018, *F. graminearum* represented 23.4% to 55.4% (mean 39.1% over 5 years) of all the *Fusarium* species isolated from 1812 wheat, 71 durum, 596 barley and 177 oat samples (Olson et al. 2019). The increased frequency or shift to *F. graminearum* has also been reported in the US, China, Brazil, Argentina, Paraguay, and Uruguay and Africa (Savary et al. 2019). Unfortunately, damage to pulse crops by *F. graminearum* has not received enough attention compared with FHB of cereals. However, the available data suggest that among pulse crops, field pea is most susceptible to *F. graminearum* (Clarkson 1978; Chongo et al. 2001; Goswami et al. 2008; Bilgi et al. 2011; Foroud et al. 2014; Rasiukevičiūtė et al. 2019).

In a previous study, the pea cultivar '00-2067' was found to possess partial resistance to Aphanomyces root rot while the cv. 'Reward' was susceptible (Wu et al. 2021). In this study, we screened the cultivars '00-2067' and 'Reward' to determine their reaction to five Fusarium isolates representing *F. solani*, *F. avenaceum*, *F. acuminatum*, *F. proliferatum* and *F. graminearum*. The cultivar '00-2067' was partially resistant to all five Fusarium species, which suggests that it might be tolerant to many pathogens of the pea root rot complex. The disease severity difference between the mean root rot values of the two cultivars was significant ($p < 0.001$) for only the isolates representing *F. avenaceum* and *F. graminearum*. Therefore, the F_8 RIL population derived from 'Reward' × '00-2067' were screened with F4A (*F. avenaceum*) and FG2 (*F. graminearum*) for the detection of partial resistance to the two *Fusarium* species. The greenhouse experiments were repeated four times, to determine the G × E interaction for all traits. In addition, the best linear unbiased predictors (BLUPs) and least square mean (LSM) were applied to minimize environmental effects (Wang et al. 2018). The LSM identified six QTL, while BLUPs identified five QTL, suggesting indicated that the LSM and BLUPs of the pooled data had comparable efficiency to detect important QTLs.

Transgressive segregation was found for disease severity in the RILs inoculated with FG2 and F4A. This suggested that different resistance loci derived from the parental cultivars might have contributed to the stronger resistance observed in some of the RILs. Some transgressive RILs, such

as X1303-19-3-1, X1303-21-3-1, X1303-26-2-1, X1304-21-3-1 and X1304-22-3-2, had lower disease severity in response to FG2 and higher vigor in all four environments compared with '00-2067'. In response to F4A, the RIL X1303-29-4-1 showed greater resistance and vigor compared with '00-2067'. Transgressive segregation was reported in other studies of resistance to Fusarium and Aphanomyces root rot in field pea (Feng et al. 2011; Mc Phee et al. 2012; Nakedde et al. 2016; Coyne et al. 2015, 2019; Wu et al. 2021). These transgressive lines will be valuable resources for developing commercial pea cultivars with improved resistance to *F. graminearum* and *F. avenaceum* and other pathogens of the pea root rot complex.

The average marker density of 1.8 marker/cM in this study was much greater than what has been reported in previous studies of pea with PCR-based markers, while the total map length (1704.9 cM) was comparable. Feng et al. (2011) constructed a linkage map of 53 cM with 14 SSR markers and obtained a marker density of 0.26 marker/cM. Mc Phee et al. (2012) constructed a linkage map of total length 1716 cM with 278 PCR-based markers and reported a marker density of 0.16 marker/cM. Similarly, Coyne et al. (2015) used 178 PCR-based markers to construct a linkage map of 1323 cM and obtained a marker density of 0.13 maker/cM. More recently, Coyne et al. (2019) applied 914 SNP markers to construct a linkage map of total length 1073 cM and reported a marker density 0.85 marker/cM. A marker density of 3.5 marker/cM and total map length of 843 cM were obtained when 18 pea lines were genotyped with the same SNP array set used in this study (Desgroux et al. 2016).

In this study, 11 QTL accounting for disease severity, vigor and plant height were identified. The major QTL for disease resistance was located on Chrom4/LGIV, while two minor QTLs were detected on Chrom5/LGIII and one QTL on Chrom3/LGV. These QTL were coincident with the QTL detected for resistance to Aphanomyces root rot (Wu et al. 2021). The major QTL (R^2= 68%-80%) identified by Mc Phee et al. (2012) for resistance to *F. oxysporum* was also located on Chrom4/LGIV, while three minor QTL (R^2= 2.8%-5.4%) were located on Chrom5/LGIII. Despite identifying the same chromosomes, the similarity of the location of the QTL cannot be confirmed given the different markers used in the two studies. However, the coincidence of the QTL is not

surprising, since very few partially resistant pea cultivars are used in breeding programs across the world. Feng et al. (2011) reported that the major QTL for root rot severity caused by *F. avenaceum* was located on Chrom/LGVII. Coyne et al. (2015, 2019) reported that the major QTL for resistance to *F. solani* was located on Chrom6/LGII, while several minor QTL were located on Chrom5/LGIII, Chrom4/LGIV, Chrom6/II and Chrom7/LGVII.

Significant QTL × QTL interactions was found between the minor QTL for disease severity and plant height but not for vigor. An interaction of the major QTL for disease severity, vigor and height was not observed. Wu et al. (2021) reported that the same genomic regions controlled disease severity and vigor, while plant height was a poor measure of Aphanomyces root rot severity in pea. Coyne et al. (2019) treated plant height as a direct disease-related trait. In contrast, Desgroux et al. (2016) considered plant height as an agronomic trait. The reduced epistatic interaction might be due to a reduction in the detected number of additive-effect QTL from 27 in Wu et al. (2021) to 11 in the current study.

To the best of our knowledge, no genetic studies have been carried out to determine the genomic regions associated with the partial resistance of field pea to *F. graminearum*. The use of high-density SNP markers and SSR anchor markers contributed to the construction of a fine linkage map and the identification of two stable QTL located on Chrom4/LGIV associated with partial resistance to *F. graminearum*. The identified QTL showed broad resistance to *F. graminearum*, *F. solani*, *F. avenaceum*, *F. acuminatum*, *F. proliferatum*, as well as *A. euteiches*. This study, and with our previous report (Wu et al. 2021), suggest that '00-2067' and the transgressive RILs with lower disease severity can be used to develop pea cultivars with improved root rot resistance.

Table 3.1. Statistical summary of phenotypic data for the parental pea cultivars, '00-2067' and 'Reward', and a RIL population inoculated with *Fusarium graminearum* isolate FG2, in four greenhouse experiments as well as the pooled and the best linear unbiased predictors (BLUPs) of the greenhouse experiments

Abbrev.	Parental cultivar			RIL population			
	'00-2067'	'Reward'	T-test (*P*)	RILs	Skewness	Kurtosis	Shapiro-test (*P*)
DSGH1	1.5±0.7	3.3±0.5	1.1E-02	2.0±0.7	-0.1	-0.7	1.6E-03
DSGH2	1.3±0.6	3.3±0.5	2.6E-03	1.9±0.7	-0.2	-0.6	1.5E-02
DSGH3	2.0±1.2	3.5±0.6	3.0E-02	2.2±0.7	0.0	-0.2	6.0E-02
DSGH4	1.5±0.7	3.0±0.0	1.3E-02	2.1±0.7	0.1	-0.3	3.7E-02
DSGHPooled	1.6±0.8	3.3±0.5	4.6E-07	2.0±0.6	-0.1	-0.7	6.2E-02
DSGHBLUPS	1.2	4.1	-	2.0±1.2	-0.1	-0.8	4.6E-02
VGH1	4.0±0.0	2.0±0.8	1.5E-02	3.0±0.8	-0.3	-0.7	0.0E+00
VGH2	4.0±0.0	2.5±0.6	3.5E-03	3.0±0.7	-0.3	-0.2	9.5E-06
VGH3	3.0±1.2	1.5±0.6	3.0E-02	2.7±0.7	-0.2	-0.2	5.1E-06
VGH4	3.5±0.7	2.7±0.6	1.2E-01	2.8±0.8	-0.4	0.6	1.1E-03
VGHPooled	3.6±0.8	2.2±0.7	6.0E-05	2.9±0.5	0.0	-0.5	1.1E-01
VGHBLUPS	4.2	1.6	-	2.9±1.1	-0.1	-0.4	5.1E-02
HGH1	234.5±54.6	177.5±36.1	1.3E-01	217.6±96.3	1.0	0.7	0.0E+00
HGH2	157.3±50.6	120.7±31.5	1.6E-01	231.5±87.4	0.7	0.2	5.6E-05
HGH3	155.5±59.5	129.5±26.0	2.3E-01	154.5±84.4	1.0	1.1	8.0E-06
HGH4	159.7±6.7	178.5±34.6	2.0R-01	184.6±83.9	0.6	0.5	5.5E-02
HGHPooled	176.7±56.6	151.5±36.9	5.1E-02	197.5±68.8	1.1	1.2	0.0E+00
HGHBLUPS	158.9	100.8		197.3±135.5	1.0	0.6	0.0E+00

Table 3.2. Statistical summary of phenotypic data for the parental pea cultivars, '00-2067' and 'Reward', and a RIL population inoculated with *Fusarium avenacium* isolate F4A, in four greenhouse experiments as well as the pooled and the best linear unbiased predictors (BLUPs) of the greenhouse experiments

Abbrev.	Parental cultivar			RIL population			
	'00-2067'	'Reward'	T-test (*P*)	RILs	Skewness	Kurtosis	Shapiro-test (*P*)
DSGH1	1.0±0.0	3.3±0.5	5.30E-05	2.2±0.9	0.2	-0.9	7.77E-05
DSGH2	1.0±0.8	3.3±0.5	1.66E-03	2.3±0.9	0.3	-0.6	1.35E-04
DSGH3	1.3±0.5	3.5±0.6	5.30E-04	2.5±0.9	0.0	-1.0	2.86E-06
DSGH4	1.0±0	3.0±0.0	1.36E-03	2.4±0.9	0.0	-0.7	1.38E-02
DSGHPooled	1.1±0.4	3.3±0.4	2.63E-13	2.4±0.7	0.0	-0.6	1.20E-01
DSGHBLUPS	1.0	3.0		2.3±0.9	0.1	-1.0	8.07E-05
VGH1	4.0±0.0	1.7±0.5	5.26E-05	2.6±1.1	-0.5	-0.6	5.96E-08
VGH2	3.7±0.5	2.0±1.4	2.92E-02	2.6±1.1	-0.9	-0.1	0.00E+00
VGH3	3.5±0.6	1.2±1.5	1.56E-02	2.4±1.2	-0.5	-0.7	0.00E+00
VGH4	4.0±0.0	2.5±0.6	1.01E-03	2.5±1.1	-0.4	-1.0	1.79E-07
VGHPooled	3.8±0.5	1.9±1.1	1.06E-07	2.5±0.9	-0.5	-0.6	9.89E-06
VGHBLUPS	4.0	1.9		2.6±1	-0.6	-0.3	5.96E-08
HGH1	210.8±128.2	118.3±100.2	1.49E-01	196.8±87.9	0.7	0.6	1.35E-03
HGH2	174.5±104.8	193.5±104.5	4.03E-01	194.5±88.5	0.6	1.3	9.74E-02
HGH3	159.5±13.5	125.0±98.9	2.58E-01	171.8±80	0.4	0.3	3.76E-01
HGH4	210.0±53.1	194.0±38.4	3.00E-01	180.8±80.1	0.7	0.7	8.17E-03
HGHPooled	188.7±82.8	157.7±88.5	1.54E-01	185.2±64	0.6	0.5	9.79E-02
HGHBLUPS	208.0	133.1		186.6±122.6	0.6	1.0	5.72E-02

Table 3.3. Summary of the QTL associated with Fusarium root rot severity, vigor and plant height in 128 F_8-derived recombinant inbred pea lines from the cross between the cultivars 'Reward' × '00-2067' in greenhouse.

Identified QTL	Trait Abbrev.	LG	Chrom[α] / LG[β]	Peak (cM)	Confidence interval(cM)	Left Marker	LOD	Additive	R^2 (%)
Fg-Ps3.1	DSGH1	4	Chrom5/ LGIII	311.9	307.9-316.5	AA5	3.9	-0.2409	9.88
Fg-Ps3.2	DSGH4	4	Chrom5/LGIII	338.2	334.9-341.4	PsCam036163_21311_1095	3.5	-0.2153	9.62
Fg-Ps4.1	DSGH1	5	Chrom4/ LGIV	71.7	63.7-74.4	PsCam050913_33466_1250	3.0	-0.2121	9.10
	DSGH2	5	Chrom4/ LGIV	61.3	59.3-69.2	PsCam001381_1152_437	3.8	-0.2229	10.57
Fg-Ps4.2	DSGH3	5	Chrom4/LGIV	80.2	74.0-85.2	AA239	5.9	-0.2344	11.26
	DSGH4	5	Chrom4/LGIV	80.2	75.4-85.2	AA239	4.1	-0.2526	13.17
	DSGHP	5	Chrom4/LGIV	79.2	75.4-85.2	AA239	5.1	-0.2492	15.44
	DSGHB	5	Chrom4/LGIV	79.2	75.4-85.2	AA239	3.7	-0.3838	10.02
Fg-Ps5.1	DSGH1	7	Chrom3/LGV	5.2	0.9-9.2	PsCam059449_39630_321	5.5	0.3036	14.22
Vig-Ps3.1	VGH4	4	Chrom5/ LGIII	68.9	67.1-70.5	PsCam013763_9362_423	4.9	-0.1423	4.05
Vig-Ps3.2	VGH2	4	Chrom5/ LGIII	312.0	307.8-316.8	AA5	3.0	0.1910	9.53
	VGH3	4	Chrom5/ LGIII	316.1	310.4-320.4	AA5	3.3	0.2582	11.22
	VGHP	4	Chrom5/ LGIII	312.6	310.4-316.5	AA5	4.6	0.1938	12.13
	VGHB	4	Chrom5/ LGIII	312.6	307.5-316.5	AA5	4.2	0.3736	11.92
Vig-Ps4.1	VGH1	5	Chrom4/ LGIV	68.0	63.5-69.9	PsCam050913_33466_1250	4.4	0.2728	13.50
	VGH2	5	Chrom4/ LGIV	60.3	58.0-70.7	PsCam000712_620_237	3.2	0.2060	10.42
	VGH4	5	Chrom4/ LGIV	71.5	70.5-73.2	PsCam042375_26443_3427	4.5	0.2437	9.19
	VGHP	5	Chrom4/ LGIV	61.3	58.8-63.5	PsCam000712_620_237	4.4	0.1868	11.59
	VGHB	5	Chrom4/ LGIV	61.3	59.3-63.5	PsCam001381_1152_437	3.8	0.3474	10.51
Hgt-Ps3.1	HGH1	4	Chrom5/ LGIII	288.6	288.3-291.7	PsCam020937_11699_2576	14.4	-62.31	36.35
	HGH2	4	Chrom5/ LGIII	287.6	286.8-293.7	PsCam020937_11699_2576	4.7	-33.90	12.90
	HGH4	4	Chrom5/ LGIII	287.6	286.8-295.2	PsCam020937_11699_2576	3.3	-27.27	9.94
	HGHP	4	Chrom5/ LGIII	287.6	286.8-292.4	PsCam020937_11699_2576	6.2	-33.24	20.96
	HGHB	4	Chrom5/ LGIII	287.6	286.8-291.4	PsCam020937_11699_2576	9.4	-71.63	23.97
Hgt-Ps7.1	HGH1	9	Chrom7/ LGVII	92.2	85.3-102.1	PsCam039854_24711_656	10.1	46.60	20.04
	HGHP	9	Chrom7/ LGVII	92.2	84.5-115.3	PsCam056683_37453_248	4.9	28.68	13.54
	HGHB	9	Chrom7/ LGVII	92.2	81.2-102.5	PsCam035831_20992_561	4.5	52.40	7.04
Hgt-Ps7.2	HGH2	9	Chrom7/ LGVII	154.3	143.8-167.5	PsCam002756_2184_427	5.1	34.63	13.63
	HGH4	9	Chrom7/ LGVII	144.3	142.3-151.9	PsCam002756_2184_427	4.4	32.77	13.85
	HGHP	9	Chrom7/ LGVII	157.7	148.8-168.0	AB91	4.4	27.87	14.06

[α] Pea chromosomes named according to Neumann et al. (2002) and [β] Pea linkage groups named according to Tayeh et al. (2015).

Table 3.4. Summary of the major and moderate digenic epistatic interactions (QTL × QTL) detected for Fusarium root rot severity, vigor and plant height in four greenhouse experiments with pea.

Epistaic-effect QTL	Trait Abbrev.	LG	QTL 1 position	Left Marker QTL 1	LG	QTL 2 position	Left Marker QTL 2	R^2 (%)	Linked additive-effect QTL
E.FG2-Ps1	DSGH1	III	130	PsCam055659_36655_1042	III	315	AA5	9.5	*Fg-Ps3.1*
E.FG2-Ps2	DSGH1	III	345	PsCam004460_3351_975	IV	175	PsCam001246_1050_252	7.8	*Fg-Ps3.2*
E.FG2-Ps3	DSGH2	II	240	PsCam052149_34531_162	III	175	PsCam005343_4052_245	12.4	-
E.FG2-Ps4	DSGH2	III	135	PsCam007018_5220_1401	III	220	PsCam035416_20603_89	10.1	-
E.FG2-Ps5	DSGH3	III	160	PsCam005343_4052_245	III	220	PsCam035416_20603_89	9.0	-
E.FG2-Ps6	DSGHP	III	170	PsCam005343_4052_245	III	215	PsCam035416_20603_89	9.2	-
E.FG2-Ps7	DSGHB	III	170	PsCam005343_4052_245	III	215	PsCam035416_20603_89	10.5	-
E.FG2-Ps8	DSGHB	III	245	PsCam042923_26960_468	III	90	PsCam005789_4353_36	7.5	-
E.Vig-Ps1	VGH1	III	175	PsCam005343_4052_245	III	375	PsCam019069_11310_393	11.2	-
E.Vig-Ps2	VGH2	I	55	PsCam037897_22954_2120	III	170	PsCam005343_4052_245	10.0	-
E.Vig-Ps3	VGH2	III	170	PsCam005343_4052_245	III	215	PsCam035416_20603_89	12.1	-
E.Vig-Ps4	VGH4	I	55	PsCam037897_22954_2120	III	190	PsCam005343_4052_245	8.5	-
E.Vig-Ps5	VGH4	III	195	PsCam005343_4052_245	III	215	PsCam035416_20603_89	8.7	-
E.Vig-Ps6	VGH4	III	185	PsCam005343_4052_245	VII	50	PsCam017623_10858_46	9.6	-
E.Vig-Ps7	VGHP	III	220	PsCam035416_20603_89	III	315	AA5	12.6	*Fg-Ps3.1*
E.Vig-Ps8	VGHP	III	390	PsCam029411_17551_1348	V	85	PsCam005789_4353_36	7.8	-

Table 3.4 continued. Summary of the major and moderate digenic epistatic interactions (QTL × QTL) detected for Fusarium root rot severity, vigor and plant height in four greenhouse experiments with pea.

Epistaic-effect QTL	Trait Abbrev.	LG	QTL 1 position	Left Marker QTL 1	LG	QTL 2 position	Left Marker QTL 2	R^2 (%)	Linked additive-effect QTL
E.Vig-Ps9	VGHP	III	215	PsCam035416_20603_89	VI	85	PsCam042529_26584_303	10.0	-
E.Vig-Ps10	VGHB	III	85	PsCam050501_33079_1023	III	170	PsCam005343_4052_245	9.8	-
E.Hgt-Ps1	HGH1	III	340	PsCam004460_3351_975	III	345	PsCam004460_3351_975	31.2	*Fg-Ps3.2*
E.Hgt-Ps2	HGH1	III	340	PsCam004460_3351_975	VII	60	PsCam001066_909_911	9.9	*Fg-Ps3.2*
E.Hgt-Ps3	HGH2	III	70	PsCam004460_3351_975	III	345	PsCam004460_3351_975	13.2	*Vig-Ps3.1* and *Fg-Ps3.2*
E.Hgt-Ps4	HGHP	III	340	PsCam042923_26960_468	III	335	PsCam004460_3351_975	13.5	*Fg-Ps3.2*
E.Hgt-Ps5	HGHP	III	340	PsCam004460_3351_975	IV	25	PsCam054029_35722_104	8.9	*Fg-Ps3.2*
E.Hgt-Ps6	HGHP	III	340	PsCam004460_3351_975	VII	60	PsCam001066_909_911	8.9	*Fg-Ps3.2*
E.Hgt-Ps7	HGHB	III	340	PsCam004460_3351_975	III	345	PsCam004460_3351_975	19.1	*Fg-Ps3.2*

Table 3.5. Root rot severity of the parental pea cultivars '00-2067' (partially resistant) and 'Reward' (susceptible) to five *Fusarium* spp. under controlled conditions in the greenhouse.

Pathogen	*F. solani*	*F. avenaceum*	*F. acuminatum.*	*F. proliferatum*	*F. graminearum*
Isolates	**S4C**	**F4A**	**F037**	**F039**	**FG2**
00-2067	3.1±1.2	1.8±0.5	0.8±0.2	2.2±0.5	1.1±0.4
Reward	3.7±0.3	2.8±0.2	1.8±0.6	3.3±0.5	3.3±0.4
T-test	0.1022	3.94E-04	1.122 E-03	0.001103	4.84E-07

Table 3.6a. Analysis of variance (ANOVA) for root rot severity, vigor and plant height using the pooled data of a RIL population of pea inoculated with *Fusarium graminearum* isolate FG2 in four greenhouse experiments.

Source of Variance	df	Mean square		
		DS	Vigor	Height
Genotype (G)	128	4.23***	3.90***	63130***
Year-station (Y-S)	3	5.25***	17.78***	213909***
Rep	3	0.96	3.51**	38462***
G*Y-S	379	0.32	0.65	7228
Residuals	934	0.57	0.67	6826
Heritability		0.92	0.86	0.91

Note: Significance difference codes: 0 '***'; 0.001 '**'; 0.01 '*'; 0.05 '.'; 0.1 ' '.

Table 3.6b. Analysis of variance (ANOVA) for root rot severity, vigor and plant height using the pooled data of a RIL population of pea inoculated with *Fusarium avenacium* isolate F4A in four greenhouse experiments

Source of Variance	df	Mean square		
		DS	Vigor	Height
Genotype (G)	129	9.34***	12.54***	63149***
Year-station (Y-S)	3	5.39***	7.82***	91477***
Rep	3	9.59***	25.56***	177623***
G*Y-S	380	0.76*	1.72***	13266***
Residuals	1327	0.64	1.21	8752
Heritability		0.92	0.86	0.79

Note: Significance difference codes: 0 '***'; 0.001 '**'; 0.01 '*'; 0.05 '.'; 0.1 ' '.

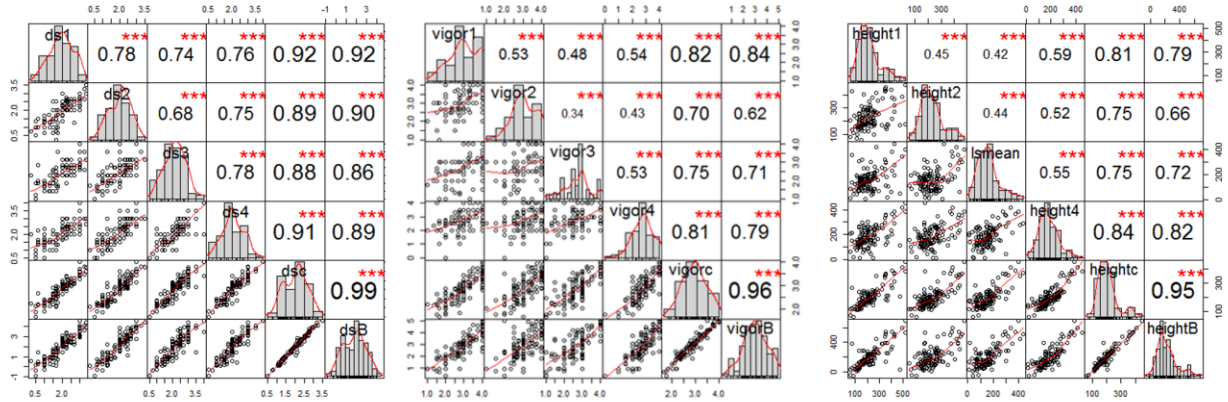


Figure 3.1. Correlation analysis of estimated mean of four single greenhouse experiments, BLUPs and combined total data for root rot severity, vigor and height of pea inoculated by FG2, illustrating the significant correlation among all variables for each trait. The bar graphs indicate the frequency distributions across the diagonal. The correlation coefficients with a significance level (* indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$) and scatter plots between pairs are shown above and below the diagonal, respectively.

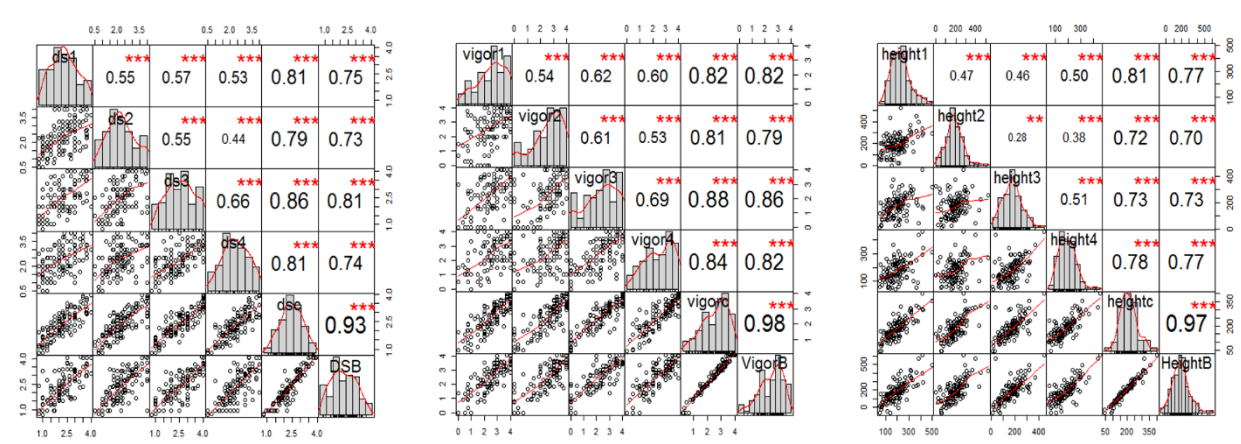


Figure 3.2. Correlation analysis of estimated mean of four single greenhouse experiments, BLUPs and combined total data for root rot severity, vigor and height of pea inoculated by F4A, illustrating the significant correlation among all variables for each trait. The bar graphs indicate the frequency distributions across the diagonal. The correlation coefficients with a significance level (* indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$) and scatter plots between pairs are shown above and below the diagonal, respectively.

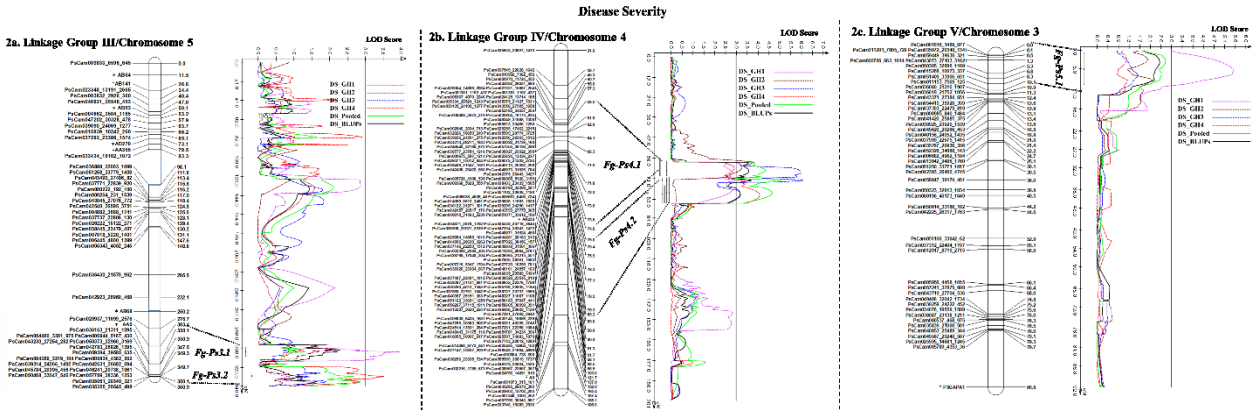


Figure 3.3. Identified QTL and linkage map of pea LG III (chrom 5), IV (chrom 4) and V (chrom 3) associated with partial resistance to *Fusarium graminearum* in an F_8 RIL derived from 'Reward' × '002067'. The LOD scores are indicated on the x-axis, while the genetic distances (in cM) are indicated on the y-axis. (a) Two minor-effect QTL, *Fg-Ps3.1* and *Fg-Ps3.2*, on LG III (chrom 5) were detected in greenhouse experiments 1 and 4, respectively. (b) Two stable, moderate-effect QTL, *Fg-Ps4.1* and *Fg-Ps4.2*, were located on LG IV (chrom 4) and identified in greenhouse experiments 1 and 2 and 3 and 4, respectively. (c) Another moderate-effect QTL, *Fg-Ps5.1*, on LG V (chrom 5) wase detected only in greenhouse experiment 1.

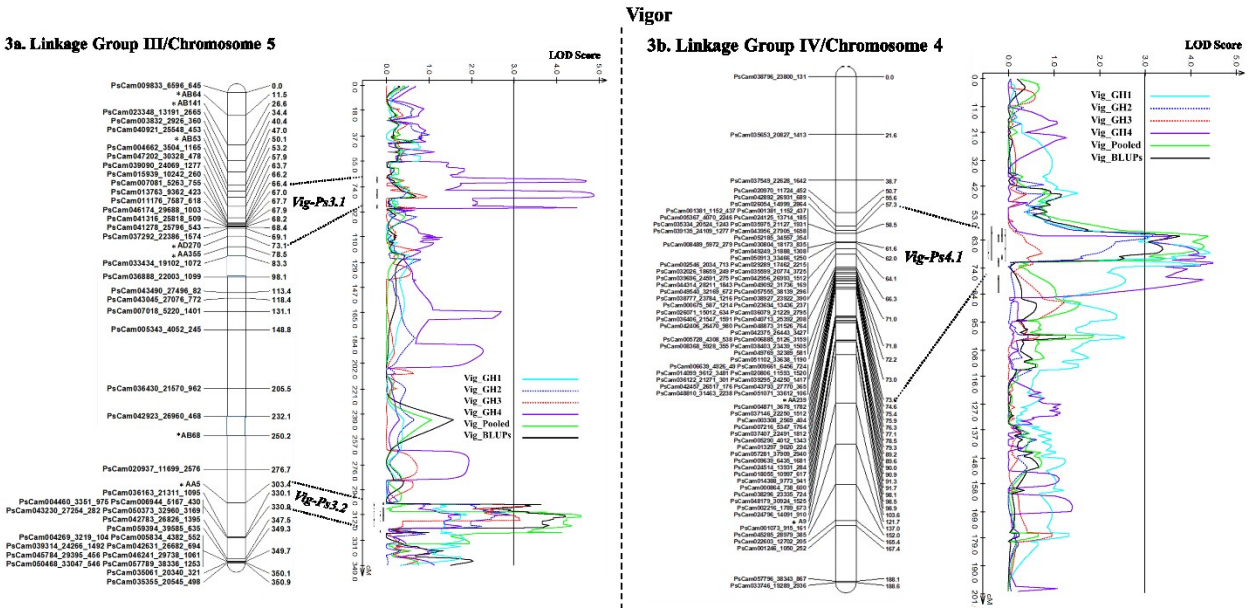


Figure 3.4. QTL likelihood profile and linkage map of pea LG III (chrom 5) and IV (chrom 4 for vigor in an F_8 RIL of the cross 'Reward' × '002067'. The LOD scores are indicated on the x-axis while the genetic distances (in cM) are indicated on the y-axis. (a) A minor-effect QTL *Vig-Ps3.1* on LG III (chrom 5) was detected for vigor only in greenhouse experiment 4. Another QTL *Vig-Ps3.2* was identified multiple times in greenhouse experiments 2 and 3, as well as in the BLUPs and pooled data (b) One minor-moderate-effect QTL, *Vig-Ps4.1*, was identified on LG IV (chrom 4) greenhouse experiments 1,2 and 5 as well as the pooled data and BLUPs.

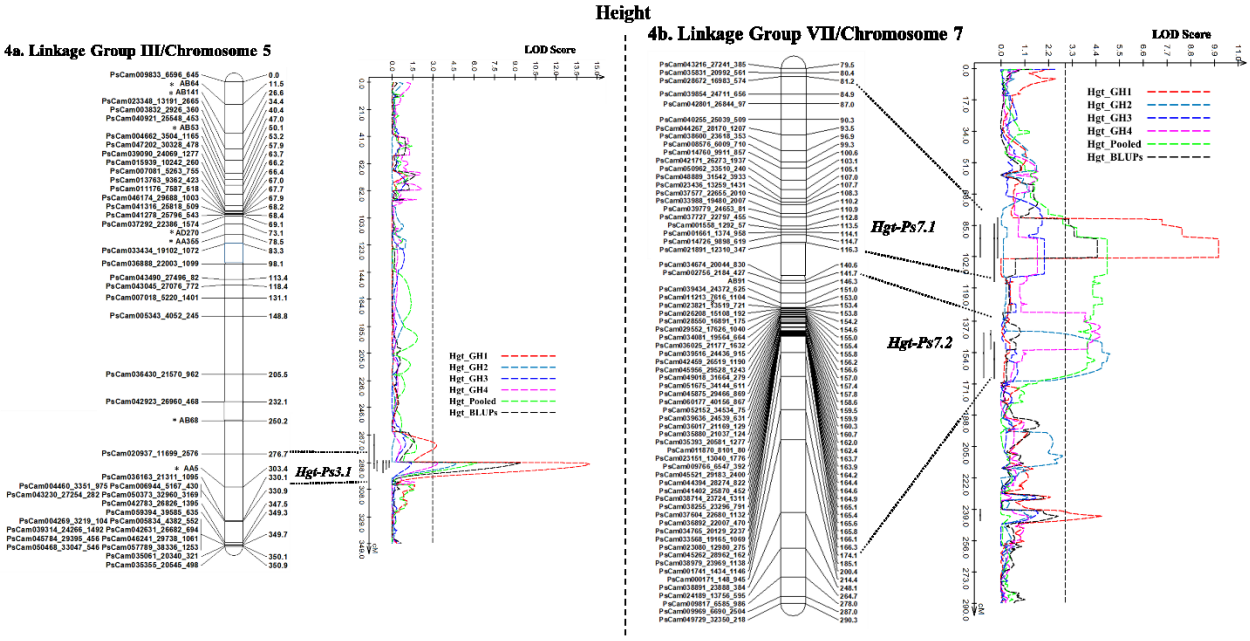


Figure 3.5. QTL likelihood profile and linkage map of pea III (chrom 5) and VII (chrom 7) for plant height in an F_8 RIL of the cross 'Reward' × '002067'. The LOD scores are indicated on the x-axis, while the genetic distances (in cM) are indicated on the y-axis. (a) One stable QTL, *Hgt-Ps3.1* on LG III (chrom 5), was detected by all variables except greenhouse experiment 3, with a minor to major effect. (b) Two QTL were detected on LG VII (chrom 7); the minor-major-effect QTL *Hgt-Ps7.1* was detected in greenhouse experiment 1 as well as in the pooled data and BLUPs, while the moderate effect QTL *Hgt-Ps7.2* was detected in greenhouse experiments 2 and 4 and the pooled data.

Chapter 4 Identification of novel genes associated with partial resistance to Aphanomyces root rot in field pea by BSR-seq analysis

4.1 Introduction

Field pea (*Pisum sativum* L.) is an economically important crop belonging to the Fabaceae family. It is characterized by high protein content in the seeds, ability to fix nitrogen and adaptation to cool seasons. These properties make field pea an ideal rotational crop in western Canada, where canola and wheat are the major crops. Canada is the largest field pea producer in the world (≈4.5 million tonnes in 2020), of which 82% is exported (Agriculture and Agri-Food Canada 2021). Unfortunately, the root rot complex, which involves several soilborne pathogens including *Fusarium* spp., *Aphanomyces euteiches*, *Pythium* spp., *Phytophthora* spp. and *Rhizoctonia* spp., is a major limitation to field pea production in Canada (Xue et al. 1998; Fletcher et al. 1991; Hwang et al. 1994; Bailey et al. 2003; Chang et al. 2005, 2013, 2014, 2017).

The oomycete *A. euteiches* is one of the most destructive pathogens of the root rot complex, infecting field pea at all stages of development. During the early stages of infection, *A. euteiches* causes damping-off and severe root rot. Later in the growing season, under favorable conditions, *A. euteiches* infection can destroy the root system completely, resulting in severe yield loss. Aphanomyces root rot (ARR) has been reported across the major pea-producing regions worldwide (Wade 1955; Yokosawa et al. 1974; Wicker and Rouxel 2001), including the Canadian provinces of Alberta and Saskatchewan (Banniza et al. 2013; Chatterton et al. 2015).

Cultural management practices and fungicidal seed treatments are insufficient to suppress ARR under field conditions (Malvick et al. 1994; Pilet-Nayel et al. 2002; Conner et al. 2013; Wu et al. 2019), and genetic resistance may be the most promising tool to manage the disease. While field pea cultivars with complete resistance to ARR are not available, genotypes with partial polygenic resistance are used for disease management. Several plant introduction (PI) lines of pea have been developed to control ARR (Shehata et al. 1983) and the pea cultivar '00-2067' was reported to be tolerant to *A. euteiches* (Conner et al. 2013; Wu et al. 2021).

Previous studies have reported several genomic regions and quantitative trait loci (QTL) associated with the partial resistance to ARR. Major QTL associated with resistance to ARR were reported on linkage group (LG) IV and VII, while minor QTL were reported on LG I, II, III and V (Pilet-Nayel et al. 2002, 2005; Hamon et al. 2011, 2013; Lavaud et al. 2015). These studies, however, used a limited number of PCR-based markers, resulting in low marker densities and relatively large QTL intervals. This makes it difficult to apply the flanking markers associated with the reported QTL for marker-assisted selection. Genotyping and mapping with high-density SNP arrays identified small-sized interval QTL associated with partial resistance to ARR in field pea (Desgroux et al. 2016; Wu et al. 2021).

Next-generation sequencing (NGS) is a revolutionary technology that has gained widespread use in crop improvement (Bolger et al. 2014). This technology can detect polymorphisms in DNA, mRNA and small RNA sequences, and elucidate transcriptional processes, splicing patterns and gene expression levels (Shaffer 2007; Shendure 2008; Kahvejian et al. 2008; Ansorge 2009; Wang et al. 2009; Ray and Satya 2014). RNA sequencing (RNA-seq) analysis has been applied in many crops, including maize (*Zea mays*) (Sekhon et al. 2013; 2014), wheat (Iquebal et al. 2019; Chu et al. 2021), alfalfa (Yang et al. 2011; Postnikova et al. 2013) and soybean (Severin et al. 2010), to detect the presence and quantity of RNA under biotic and abiotic stress. Recent RNA-seq analyses of field pea have focused on the study of seed development (Liu et al. 2015), agronomic characters (Sudheesh et al. 2015), root nodulation (Alves-Carvalho et al. 2015) and arbuscular mycorrhizal (AM) symbioses (Afonin et al. 2020). Bulked segregant RNA-sequencing (BSR-seq) technology combines NGS technology and bulked sergeant analysis (Hu et al. 2018), and has been used for the identification of gene-related markers associated with disease resistance in maize, wheat and canola (Liu et al. 2012; Yu et al. 2016; Hu et al. 2018; Wu et al. 2018). Liu et al. (2012) detected novel polymorphic markers associated with the 'glossy' (gl3) phenotype in a small-sized interval, leading to the cloning of this gene. BSR-seq was also used for molecular characterization of the resistance genes *Yr15*, *YrZH22, YrMM58*, *YrHY1*, *Yr26*, *Pm4b* and *PmSGD*

in wheat (Hu et al. 2018), and to identify the clubroot resistance gene *Rcr1* in canola and for the identification of markers for marker-assisted selection (Yu et al. 2016).

At present, an increasing number of pathway databases are available for exploration of visualized biological mechanisms with associated open reading frames (ORFs), genes and proteins, including the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa 2000), Plant Reactome (Naithani et al. 2016), MetaCyc (Caspi et al. 2014) and others. Many legume crops, not including field pea, are available in the KEGG database, which is usually used for annotation of pea nucleotide sequences against other available legume crops, such as chickpea, soybean and *Medicago truncatula* (Alves-Carvalho et al. 2015; Hosseini et al. 2015; Sudheesh et al. 2015). In addition to biological pathway databases, the gene ontology (GO) consortium has been developed to help evaluate the roles of genes and gene products (Ashburner et al. 2000). The GO terms contain three components: cellular components, molecular functions and biological processes, of which GO biological process are similar to the KEGG pathway, but focus on the molecular events of a gene, rather than a gene network (Nguyen et al. 2015). Currently, sequence blast, biological pathway and GO annotation are available for field pea in the Pulse Crop Database (www.pulsedb.org/).

Plant defense mechanisms associated with the interaction between field pea and *A. euteiches* are still not clear. Generally, plants initiate pattern-triggered immunity (PTI) by recognizing pathogen-associated molecular patterns (PAMPs) (Zipfel 2009). When pathogens produce effectors to suppress PTI, plants can recognize the special effectors to activate effector-triggered immunity (ETI) (Jones and Dangl 2006). Jasmonic acid (JA), ethylene (ET) and salicylic acid (SA) play important roles in the plant immune response, with the genes controlling these signaling pathways often evaluated in studies of plant defense mechanisms (Okubara and Paulitz 2005; Hosseini et al. 2015; Jewell et al. 2019). In addition, abscisic acid, auxin, brassinosteroids and gibberellins can also be involved in plant defense signaling (Robert-Seilaniantz et al. 2007).

Molecular studies of field pea have lagged behind other pulses due to its large genome size (4.45 Gb; 2n =14) and the highly repetitive nature of the genome (Smýkal et al. 2012; Kreplak et

al. 2019). In pea, RNA-seq has been used only to evaluate transcriptional gene expression levels during the interaction between field pea and *Rhizobium* (Alves-Carvalho et al. 2015; Sudheesh et al. 2015). Some *de novo* assembly studies also used RNA-seq analysis to evaluate the transcriptome of pea seed development (Liu et al. 2015; Malovichko et al. 2020). Disease-related markers in genomic regions associated with resistance to ARR are essential for marker-assisted selection. The objectives of this study were to: (1) confirm the candidate interval for resistance to ARR through BSR-seq analysis; (2) develop SNP markers to fine map the QTL associated with root rot resistance in '00-2067'; and (3) identify differentially expressed genes and predict the pathway(s) associated with resistance to *A. euteiches*.

4.2 Materials and Methods

4.2.1 Plant material

The semi-leafless parental cultivar '00-2067', derived from the crosses (PH14-119×DL-1)7 × (B563-429-2 × PI 257593) × DSP-TAC, produces white flowers and a wrinkled seed coat, and was reported to be tolerant to ARR and *Fusarium* spp. (Conner et al. 2013; Wu et al. 2021, 2022). The pedigree of the susceptible cultivar 'Carman', which produces white flowers and green cotyledons, is unknown. An F_8 RIL population was generated by single-seed descent (SSD) from the parents 'Reward' and '00-2067' and was comprised of 135 individuals.

4.2.2 Root rot assessment

Greenhouse studies were carried out in a randomized complete block design with 12 replicates. Seeds of the RIL were sterilized in 1% NaClO for 1 min and washed three times in sterilized water. Four seeds of each RIL were germinated on moistened filter paper in a Petri dish and then transplanted into 7 cm × 7 cm × 10 cm plastic pots containing sterilized nutrient soil mixture (Cell-TechTM, Monsanto, Winnipeg, MB). An *A. euteiches* (isolate Ae-MRDC1) oospore suspension was produced following Wu et al. (2021) and adjusted to a final concentration of 1×10^5 oospores mL^{-1}. Before the rootlets of the transplanted seedlings were covered with the soil mixture, each seedling was inoculated with a 1 mL aliquot of the oospore suspension. The plants were maintained

under a 12-h photoperiod at day and night temperatures of 22-28ºC and 15-18ºC, respectively. After three weeks, plant height, dry foliar weight, disease severity (DS) (0-9) and vigor (0-4) were evaluated following Wu et al. (2021). The greenhouse studies were repeated three times.

4.2.3 Bulks construction and RNA extraction

Resistant (R) and susceptible (S) bulks were generated from RILs exhibiting extreme and stable disease reactions to *A. euteiches*. Twenty-five RILs with stable resistance (DS<2.5) to *A. euteiches* and 25 RILs with stable susceptibility (DS>5.5) were selected to form the R and S bulks, respectively. About 1 cm of the main root tissue from each of the 25 individuals from each bulk was excised and mixed for RNA extraction. Each bulk contained three biological replicates. The mixed root tissues of each replicate from each bulk were ground into a powder in liquid nitrogen; the RNA was extracted from the powdered root tissue following as described by Zhou et al. (2020). Briefly, 0.1 mL root powder was homogenized in 1 mL Trizol (Ambion-Life Technologies, Carlsbad, CA, U.S.A.) for 15 min, treated with 0.2 mL chloroform (Fisher Chemical, Fair Lawn, NJ, U.S.A.) for 10 min, and precipitated using 0.5 mL 2-propanol (Fisher Chemical, Fair Lawn, NJ, U.S.A.) for 3 h. The extracted RNA was cleaned using an RNeasy Mini Kit (Qiagen, Hilden, Germany) and the DNA component of the RNA sample eliminated by treating with DNAse (Qiagen, Hilden, Germany) for 15 min at room temperature. The RNA concentration of each sample was measured in a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.) and adjusted to 50 ng/µL. An Agilent 2200 TapeStation system (Agilent, Santa Clara, CA, U.S.A.) was used to confirm the quality and purity of each RNA sample.

4.2.4 RNA-seq and sequence alignment

The cDNA library was prepared using an Illumina TruSeq stranded mRNA kit (Illumina; San Diego, CA, USA) and sequenced with a NovaSeq (Illumina). Sequence alignments were performed using a STAR (v2.7.3a) aligner and the paired-end reads were aligned to the reference *P. sativum* (ea) genome downloaded from: https://urgi.versailles.inra.fr/download/pea/Pisum_sativum_v1a.fa. The generic feature format (GFF) file was downloaded from:

https://urgi.versailles.inra.fr/download/pea/Pisum_sativum_v1a_genes.gff3). Reads that mapped to ribosomal RNA and the mitochondrial genome were removed before performing alignment. The raw read counts were estimated using HTSeq v. 0.11.2. Read counts were normalized using the package 'DESeq2' (Love et al. 2014) and hierarchical clustering analysis was performed for the normalized counts. Euclidean distance and the complete linkage clustering method were used for hierarchical clustering. Analysis was performed using R v. 3.5.2 and the additional packages: ggplot2, reshape2 and ggrepel.

4.2.5 Identification of variants between R and S bulks

Genohub Inc. (Austin, USA) generated VCF files to capture the variants for each sample of R and S bulks. The SNP and biallelic SNP numbers were obtained using the package 'SNPRelate' (Zheng et al. 2012) in R v. 3.5.2. The variants found were then used to identify SNPs with the R package 'pegas' (Paradis 2010). To improve the accuracy of statistics, the bulk comparisons for polymorphic SNP were conducted using three single R-S pairs as described by Yu et al. (2016) (R1-S1, R2-S2 and R3-S3), as well as two *in silico* mixes as described by Ricardo et al. (2015), including (i) two clustered S bulks (S2 + S3) and two clustered R bulks (R1 + R3) based on principal component analysis (PCA) and hierarchical clustering analysis (Supplementary Figure 1A, B) and (ii) all the susceptible (S1 + S2 + S3) and resistant bulks (R1 + R2 + R3). The common variants within biological replicates in the R and S bulks for the two *in silico* mixes, respectively, were detected with the R packages 'RCurl' (Lang 2022), 'purrr' (Henry and Wickham 2020), 'VariantAnnotation' (Obenchain et al. 2014), 'GenomicRanges' (Lawrence et al. 2013) and 'Rsubread' (Liao et al. 2019). Comparisons of common variants between the R and S bulks for the three single bulked pairs and two *in silico* mixes were conducted using the R package 'plyr' (Wickman 2011) to obtain monomorphic and polymorphic SNPs. The polymorphic SNP density distribution was generated with the R package 'rMVP' (Yin et al. 2021).

4.2.6 Disease-related gene expression analysis

The aligned reads were used for the estimation of the expressed genes. The raw read counts were

estimated using HTSeq (v0.11.2). The script HTseq-count is a tool for RNA-seq data analysis. The SAM/BAM file and a GTF or GFF file with gene models were used to count the number of aligned reads for each gene that overlapped with exons. Only reads that mapped unambiguously to a single gene were counted, whereas reads that aligned to multiple positions or overlapped with more than one gene were discarded. Read count data were normalized using DESeq2. Additionally, the expression of the aligned reads was estimated using cufflinks v. 2.2.1. The expression values were reported in fragments per kilobase per million (FPKM) for each gene. The significance of differentially expressed genes (DEGs) between the R and S bulks was determined based on the log2 fold change (|log2 FC|>2) for the three single bulk pairs. To better account for the variant component of the two *in silico* mixes, DEGs were also selected using the package 'DESeq2' in R v. 3.5.2 (P_{adj}<0.05). The accessions of selected genes were used in searches with BlastN and to search gene ontology (GO) terms and pathways in the Pulse Crop Database (www.pulsedb.org/) to determine gene function and biological processes, as well as associated pathways involved in the disease response.

4.3 Results

4.3.1 Root rot severity and growth parameters

ANOVA indicated a significant genotypic effect on ARR severity, vigor, plant height and dry foliar weight, suggesting that a high portion of heritable variance was transmitted from the parental cultivar to the RIL population (Table 1). Significant differences between the parental cultivars 'Carman' and '00-2067' were detected for all traits except dry foliar weight, with estimated means and stand error (SE) of 6.72 ± 1.9 and 2.2 ± 1.3 for disease severity, 1.7 ± 1.1 and 3.3 ± 0.6 for vigor, 8.0 cm ± 4.8 cm and 19.7 cm ± 5.1 cm for height, and 1.5 g ± 0.7 g and 1.3 g ± 0.6 g for dry foliar weight, respectively. Disease severity was negatively correlated with plant height, vigor and dry foliar weight, which indicated the adverse impact of ARR on overall plant growth. High correlation coefficients among the means from three greenhouse studies were found for disease severity (0.51<r<0.58, P<0.001), vigor (0.48<r<0.60, P<0.001), plant height (0.72<r<0.82,

P<0.001) and dry foliar weight (0.42<r<0.72, *P*<0.001), illustrating the stable reaction of the RIL population to ARR (Figure 4.1). The individuals used to generate the bulks were selected based on extreme scores for disease severity. The highly resistant lines (DS<2.5) constituted 33% of the total RIL population, while the highly susceptible lines represented 22% of the population.

4.3.2 RNA-seq analysis and sequence alignment

The RNA-seq analysis generated 44,595,510 - 51,658,688 and 43,848,192 - 47,866,574 raw read pairs for the three replicates of R and S bulks, respectively. The Q≥30 values ranged from 93.0% to 93.9%, which suggested high quality and accurate sequencing data. In addition, 98.1-99.4% of the reads for the R bulks were aligned to the field pea reference genome, Pisum_sativum_v1a.fa (https://urgi.versailles.inra.fr/download/pea/Pisum_sativum_v1a.fa), compared with 99.0-99.5% of the reads for the S bulks. Furthermore, 83.4-85.1% of the reads for the R and S bulks were exonic, which indicated that high portions of the tested sequences were located in the gene-encoding region. The expression level of 44,756 genes was evaluated, 56.8-57.4% of which were expressed in the R bulks and 56.8-57.3% of which were expressed in the S bulks.

4.3.3 Selection of differentially expressed genes

With a threshold of |log2 FC|>2, three single R-S pairs selected 601, 1416 and 977 DEGs for R1-S1, R2-S2 and R3-S3, respectively. By taking advantage of the DESeq analysis using the Wald test, significances were detected in 44 and 21 DEGs for the two *in silico* mixes of (R1+R3) vs. (S2+S3) and (R1+R2+R3) vs. (S1+S2+S3), respectively. Therefore, DESeq analysis determined 46 DEGs, of which 25 DEGs were down-regulated and 21 were up-regulated in the R mixed bulks compared with the S mixed bulks (Figure 4.2 and 4.3). A total of 2726 DEGs were identified by the R and S bulks comparison using either single R-S pairs or two *in silico* mixed bulks, which were located on the seven pea chromosomes: chr1LG6 (316), chr2LG1 (230), chr3LG5 (304), chr4LG4 (332), chr5LG3 (423), chr6LG2 (368) and chr7LG7 (383) (Figure 4.3).

4.3.4 Identification of variants between the R and S bulks

Frequent variants were identified for the six samples, of which the R bulks contained 238.9-254.9

K SNPs while the S bulks contained 234.4- 265.6 K SNPs. Biallelic unique SNPs detected in the R bulks consisted of 89.6-89.7% (214.4- 228.5K) of the total SNPs. A similar percentage (89.5-89.6%; 209.9-238.0 K) of the SNPs in the S bulks were biallelic unique. The polymorphic SNPs were selected for three individual R-S bulk pairs, numbering 14.9 K (R1 vs. S1), 14.6 K (R2 vs. S2) and 15.6 K (R3 vs. S3). For the two *in silico* mixes, the numbers of common SNPs within the R bulk were 160.3 K (R1+R3) and 138.3 K (R1+R2+R3), while the numbers for the S bulk mixes were 151.9 K (S2+S3) and 136.9 K. For the bulk mixes with two clustered replicates, the comparison of common SNPs between the R and S bulks identified 120.9 K (63.2%) monomorphic SNPs and 70.4 K (36.8%) polymorphic SNPs. For the bulk mixes with all three replicates, monomorphic and polymorphic SNPs were 107.7 K (64.3%) and 59.7 K (35.7%). Overall, 344.1 K polymorphic SNPs were identified based on the R and S comparison of three single R-S bulk pair and two *in silico* mixes, of which 296.6 K were aligned to seven chromosomes of field pea. The SNP densities of each chromosome were 103.7 SNPs/Mb on chromosome 1 (LGVI), 104.3 SNPs/Mb on chromosome 2 (LGI), 98.1 SNPs/Mb on chromosome 3 (LGV), 120.2 SNPs/Mb on chromosome 4 (LGIV), 10.9 SNPs/Mb on chromosome 5 (LGIII), 96.5 SNPs/Mb on chromosome 6 (LGII) and 123.9 SNPs/MB on chromosome 7 (LGVII). The most frequent variant regions were centered on the top and middle of chromosome 2 (LGI), bottom of chromosome 3 (LGV), middle of chromosome 5 (LGIV), top of chromosome 6 (LGII) and bottom of chromosome 7 (LGVII) (Figure 4.4)

4.3.5 Functional enrichment analyses of differentially expressed genes

The 2356 selected DEGs that were alignedon seven pea chromosomes were used to search GO terms associated with disease response and root growth, as well as pathways related to JA, ET and SA signaling in the Pulse Crop Database (Figure 4.5). Thirty DEGs were linked to GO biological process associated with the plant defense response, including GO:0006952, GO:0031347, GO:0031348 and GO:0031349. Meanwhile, three DEGs were associated with the plant immune response (GO:0006955), which were coincidently present in the defense-response-related DEGS. In addition, three DEGs were annotated to the GO biological process of root development,

including GO:0010015, GO:0010053, GO:0022622 and GO:0048364. For those DEGs related to the plant defense pathway, 8, 1 and 2 DEGs were involved in jasmonic acid biosynthesis, ethylene biosynthesis I and methyl-salicylate metabolism, respectively. A BlastN search of the Pulse Crop Database indicated that the 30 defense-response-related DEGs were associated with molecular functions including protein binding, ADP binding, abscisic acid binding, protein phosphatase inhibitor activity and signaling receptor activity. The DEGs Psat1g156800, Psat1g156920, Psat1g157160 Psat2g013520, Psat3g126600 and Psat4g025040 were related to the biological process of signaling defense response. All eight DEGs linked to jasmonic acid biosynthesis were annotated to the oxidation-reduction process. Jasmonic acid biosynthesis was not only related to signals that stimulated plant defenses against pathogens, herbivory, wounding and abiotic stress, but also controlled plant developmental processes such as root elongation. Both DEGs related to methyl-salicylate metabolism were associated with hydrolase activity.

4.3.6 Analysis of differential expressed genes and SNPs in the target region

The assessment of polymorphisms in the R and S bulks idenitfied a range of variants among the 44 selected DEGs on seven pea chromosomes. Three-hundred ninety five SNPs were detected within 31 annotated DEGs. In contrast, no SNPs were detected for 13 DEGs, including Psat1g110880, Psat1g156920, Psat4g087360, Psat4g201600, Psat5g066680, Psat5g242440, Psat5g242600, Psat5g289880, Psat5g291280, Psat5g291320, Psat6g011200, Psat6g098320 and Psat6g164080. Psat3g074240 contained the most (50) variants and a density of 15.2 SNPs/Kb, while Psat7g067840 included 11 SNPs but showed the highest SNP density (20.2 SNPs/Kb).

In a previous study (Wu et al. 2021), we found that the major QTL associated with partial resistance to *A. euteiches* in the pea '00-2067' was located on chromosome 4 (LG IV), while several minor to moderate effect QTLs were located on chromosomes 5 (LG III), 6 (LG II) and 7 (LG VII). In the current study, 10 of the 44 annotated DEGs were located in genomic regions reported by Wu et al. (2021). Eight of the DEGs, Psat4g152600, Psat4g180200, Psat4g180800, Psat4g184760, Psat4g185080, Psat4g186560, Psat4g201520, Psat4g201600, were located in the most stable genomic regions *AeMRDC1-Ps4.1* and *AeMRDC1-Ps4.2* reported to be associated with ARR

resistance (Wu et al. 2021). In contrast, Psat3g069000 and Psat3g074240 were located in the minor effect QTL *Hgt-Ps5.1*. The polymorphic SNP marker PsCam027331_15987_254 in the genetic map constructed by Wu et al. (2021) was annotated to Psat4g186560. In this study, 115 SNPs were found within the eight DEGs on chromosome 4, ranging from 0 to 46. For the DEGs in *Hgt-Ps5.1*, 14 and 50 SNPs were detected within Psat3g069000 and Psat3g074240, respectively. These SNPs provided a promising source of markers to merge the gap in the previous genetic map. The remaining 34 DEGs were not reported in the previous study, with 216 SNPs detected in 22 novel genes on the seven pea chromosomes.

The comparison of the R and S bulks in this study identified 250 polymorphic SNPs on all seven pea chromosomes in the DEG regions related to the defense response, of which 240, 21, 11 and 10 SNPs, respectively, were linked to GO:0006952 (29 DEGs), GO:0006952 (4 DEGs) and GO:0031348 (3 DEGs). Only one of three DEGs associated with root system development, Psat5g007800, contained 10 SNPs. In the case of the signaling defense response involving DEGs on chromosomes 2, 3, 4 and 6, a total of 45 polymorphic SNPs were detected in Psat2g149200 (13 SNPs), Psat3g069000 (14 SNPs), Psat4g184760 (46 SNPs) and Psat4g185080 (22 SNPs). However, there were no polymorphic SNPs in the other four DEGs on chromosomes 5 and 6. Sixteen and 24 SNPs were identified in Psat1g105280 and Psat3g026920, respectively, which participated in the SA signaling pathway. There was no polymorphic SNP relating to the ethylene signaling pathway.

4.4 Discussion

Aphanomyces root rot caused by *A. euteiches* is a major limitation to field pea production and has attracted significant attention from researchers in recent years. The use of partially resistant cultivars is the most effective method to control this disease, particularly given the lack of fully resistant genotypes. The pea cultivar ‘00-2067’ was reported to be partially resistant to infection by *A. euteiches* under field conditions (Conner et al. 2012), and this partial resistance to ARR as well as to Fusarium root was further explored in recent studies (Wu et al. 2021, 2022). The most stable and major QTL for resistance to the root rot complex were mapped to two genomic regions on chromosome 4, while minor to moderate QTL were located on chromosomes 5, 6 and 7 (Wu et

al. 2021, 2022).

The QTL identified by Wu et al. (2021, 2022) were determined using an F_8 RIL population derived from the cross '00-2067' (root rot-resistant parent) × 'Reward' (susceptible parent). To study the inheritance of the identified QTLs in a different genetic background, we crossed the ARR and Fusarium root rot resistant parent '00-2067' with the susceptible cultivar 'Carman' and developed RIL of 135 individuals. The results of the current study validated the stability of genetic resistance in '00-2067'. Similar to our previous studies (Wu et al. 2021, 2022), significant genotypic effects, a high correlation coefficient within each trait and a negative correlation of root rot severity with vigor and plant height were observed in the RIL population derived from '00-2067' × 'Carman'. The frequency distribution of the disease severity data for the RIL population suggested that the resistance in '00-2067' was transferred to the progenies in the RIL lines. This confirms the potential of '00-2067' as a resistance source for pea breeding programs focused on root rot diseases. Several studies have evaluated the polygenetic resistance to ARR in field pea. The QTL associated with partial resistance to *A. euteiches* were identified using PCR-based markers. However, the limited number of markers, low marker density and lack of background gene information makes it difficult to apply the identified markers for use in MAS (Pilet-Nayel et al. 2002, 2005; Hamon et al. 2011, 2013; Lavaud et al. 2015). Next-generation sequencing technology has accelerated the development of many SNPs and other markers based on gene-encoding sequences in field pea genome (Tayeh et al. 2015). These large numbers of markers can facilitate fine mapping of QTL and, more importantly, the candidate genes associated with resistance to *A. euteiches* (Desgroux et al. 2016; Wu et al. 2021). Tayeh et al. (2015) developed a pea SNP array based on agronomic traits. Genetic studies by Desgroux et al. (2016) and Wu et al. (2021) used the pea SNP array to identify QTLs associated with ARR.

RNA-seq technologies could provide a deeper understanding of gene function, regulatory networks, and the associated biological processes and pathways of the target traits, such as agronomic characters and plant defense mechanisms (Alves-Carvalho et al. 2015; Sudheesh et al. 2015; Zhu et al. 2013). The information at the genome and transcriptome levels can be used to

detect novel genes related to the target traits. Indeed, RNA-seq analysis has been applied to characterize the transcriptomes of several major crop species, including maize, wheat and soybean (Severin et al. 2010; Trapnell et al. 2012; Sekhon et al. 2013,2014; Kumar et al. 2015). Transcriptomic evaluations of field pea based on RNA-seq analysis have revealed genes associated with biological processes such as nodulation, nitrogen fixation and the plant immune response (Alves-Carvalho et al. 2015; Hosseini et al. 2015; Sudheesh et al. 2015).

Study of the field pea genome has lagged behind that of other legumes due to its large size and complexity. As such, gene function studies in field pea were usually obtained by comparison with *Medicago truncatula* (barrel clover), *Cicer arietinum* (chickpea), *Glycine max* (soybean) and *Arabidopsis thaliana* (Alves-Carvalho et al. 2015; Hosseini et al. 2015; Sudheesh et al. 2015). The pea reference genome was first published in 2019, along with abundant gene function and metabolism pathway information (Kreplak et al. 2019).

BSR-seq analysis, which can rapidly and economically detect target traits, is an improvement over RNA-seq that has been applied in maize and wheat (Liu et al. 2012; Wu et al. 2018). To our knowledge, this is the first study of its kind where BSR-seq has been used to detect novel genes in field pea. In this study, 4.3-5.1 Gb read pairs obtained from all R and S bulks were used for genome assembly, of which 98.1-99.5% were aligned to the reference genome, with about 84% of the reads located in exonic regions of the pea genome. Therefore, the results are comparable to those reported by Kreplak et al. (2019) for the pea reference genome. Sudheesh et al. (2015) applied *de novo* assembly and identified around 140 K contigs in field pea. However, they reported that only 50% configs were annotated, mostly to *M. truncatula* and soybean. Only 3.3% of the contigs matched to the gene-encoding sequences in pea. Malovichko et al. (2020) also conducted *de novo* assembly without the pea reference genome, producing 25,756 contigs distributed between 2,112 genes, much less than the 44,756 genes evaluated in the current study. The improvement of sequence matching in this study largely reflected the availability of the pea reference genome.

Eight of the DEGs associated with jasmonic acid biosynthesis in this study have been reported to be involved in oxidation-reduction processes, which can play important roles in the

plant immune response (Frederickson Matika et al. 2014; Das et al. 2015; Bleau and Spoel 2021). Seven of the eight DEGs involved in oxidation-reduction, with the exception of Psat6g098320, have been reported to control the reaction: linolenate + oxygen → 13(S)-HPOTE, which is a key step in jasmonic acid biosynthesis. Jasmonic acid plays an essential role in plant growth and development, as well as in the plant immune response (Okada et al. 2015; Ruan et al. 2019). Two DEGs involved in methyl salicylate metabolism were both annotated to the GO gene function of hydrolase activity, which can play an important role in plant defense responses by regulating ADP-ribose and NADH (Gunawardana et al. 2009). Only one DEG was linked to ethylene biosynthesis. Ethylene biosynthesis is essential in regulation of lesion mimic mutant vad1-1, which related to propagative hypersensitiveness. (Bouchez et al. 2007).

Eight selected genes were mapped to the most stable QTL region, *AeMRDC1-Ps4.1* and *AeMRDC1-Ps4.2*, associated with partial resistance to ARR (Wu et al. 2021), while two genes mapped to the minor QTL *Hgt-Ps5.1*. The 34 other genes in this study were novel and mapped to chromosomes 1, 2, 3, 4, 5 6 and 7. Previously, using a 13.2K SNP array, we mapped the major QTL for partial resistance to ARR to chromosome 4 and minor to moderate QTL to chromosomes 5, 6 and 7 (Wu et al. 2021). The present BSR-seq analysis led to the identification of novel genes associated with the partial resistance of '00-2067' to ARR, complementing the earlier work.

A total of 344.1 K SNPs were found to be polymorphic between the R and S bulks. The mean SNP density on the chromosomes ranged from 96.5 to 120.2 SNPs/Mb, except for chromosome 5, which had a very low density of 10.9 SNPs/Mb (Figure 4.4). The low variant density on this chromosome suggests that this genomic region was conserved in the population used in this study. Large intense regions of polymorphic variants with SNP densities greater than 500 SNPs/1Mb were found on chromosomes 2, 4 and 7, along with narrow intense regions on the bottom of chromosome 3 and top of chromosome 5 (Figure 4.4). This suggests that the SNPs identified in this study could be valuable in the detection of novel QTL in '00-2067'controlling resistance to ARR.

Table 1. ANOVA for root rot severity, vigor, dry foliar weight and plant height using the pooled data of a recombinant inbred line (RIL) population of pea inoculated with *Aphanomyces euteiches* in three greenhouse experiments.

Source of Variance	df	Mean square			
		DS	Vigor	Height	DFWT
Genotype (G)	134	95.1***	19.1***	1303.2***	0.069***
Repeat	2	1298.7***	192.8***	5701.6***	0.562***
G*Repeat	266	22.4***	4.2***	122.1***	0.018***
Residuals	3484	5.3	1	41.2	0.006
Heritability		0.77	0.74	0.85	0.75

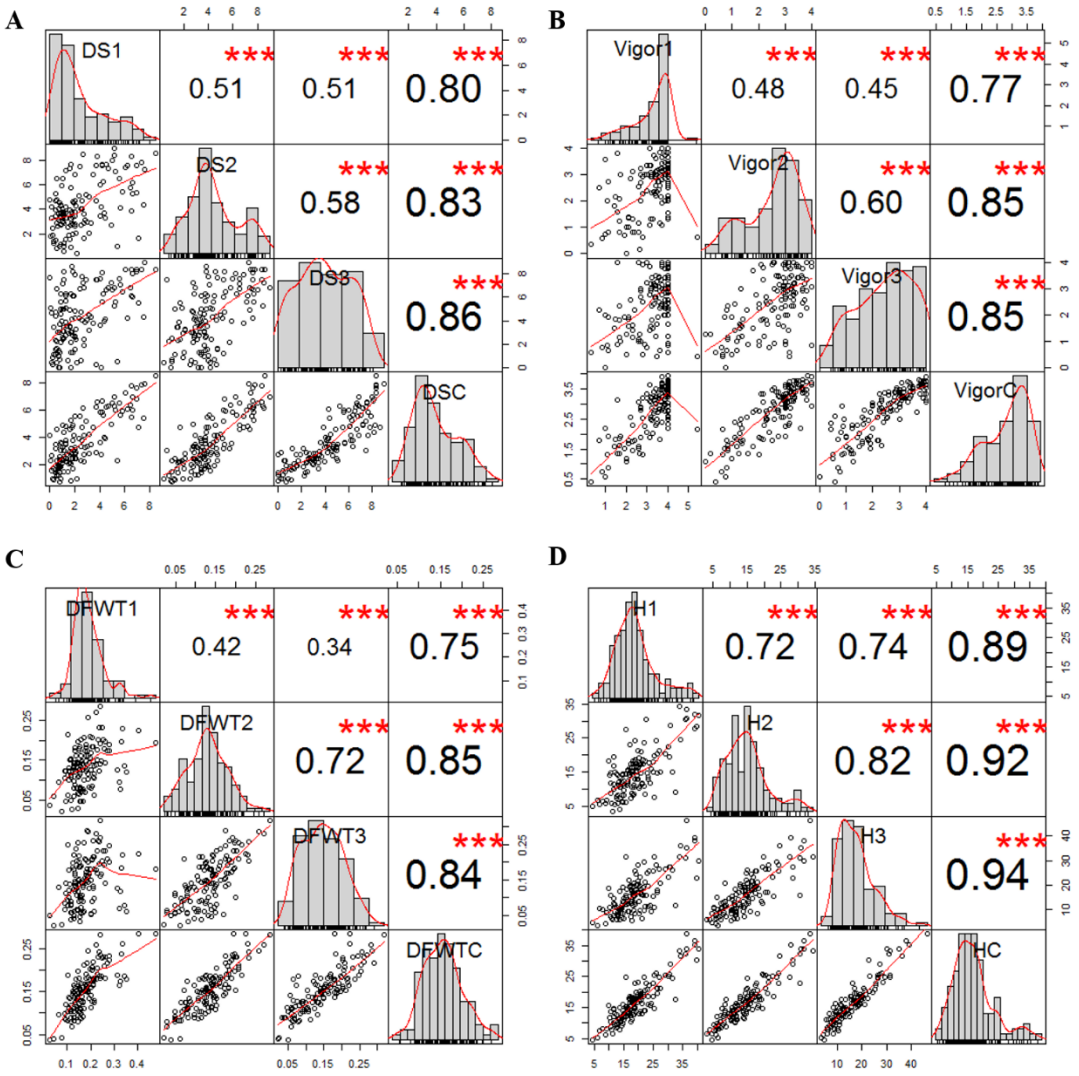


Figure 4.1. Correlation analysis of estimated mean of three single greenhouse experiments, and combined total data for (A) root rot severity, (B) vigor, (C) dry foliar weight and (D) height of pea inoculated with *Aphanomyces euteiches*, illustrating the significant correlation among all variables for each trait. The bar graphs indicate the frequency distributions across the diagonal. The correlation coefficients with a significance level (* indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.001$) and scatter plots between pairs are shown above and below the diagonal, respectively.

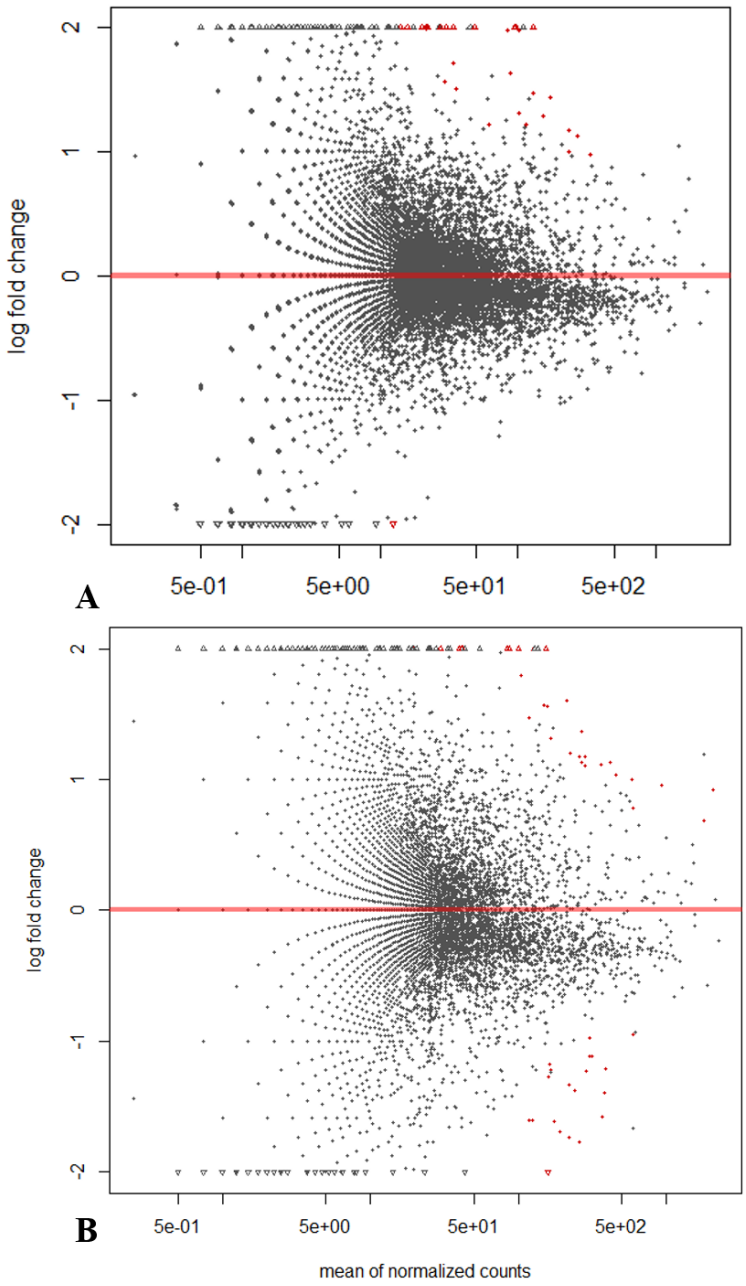


Figure 4.2. MA-plot from base means (x-axis; 'M') and the average of log fold changes (y-axis; 'A') indicating differentially expressed genes in pea resistant (R) or susceptible (S) to Aphanomyces root rot in DESeq analyses of (A) an *in silico* mix with three replicates in resistant (R) and susceptible (S) bulks; and (B) an *in silico* mix with two replicates in R and S bulks. Red spots indicate genes with $P_{adj}<0.05$.

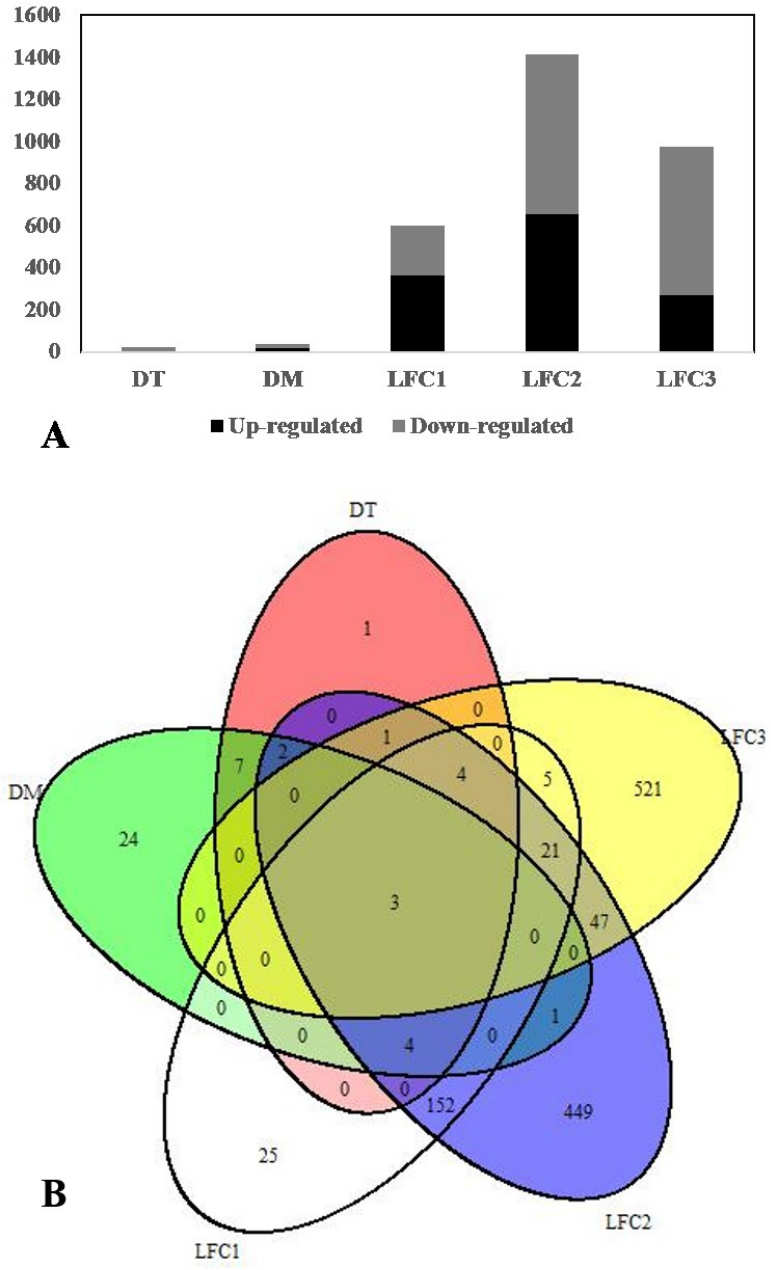


Figure 4.3. Number of differentially expressed genes (DEGs) in pea resistant (R) or susceptible (S) to Aphanomyces root rot, as detected by two *in silico* mixes and log2 fold change comparisons, as well as the overlap among these DEGs. (A) Overview of the number of significantly up-regulated and down-regulated genes. (B) The overlap in DEGs in a Venn diagram. 'DT' indicates DEGs determined by DESeq analysis from an *in silico* mix with three replicates; 'DM' indicates DEGs determined by DESeq analysis from an *in silico* mix with two replicates; and 'LFC1-3' indicates log2 fold change comparison of individual R-S pairs (R1-S1, R2-S2 and R3-S3).

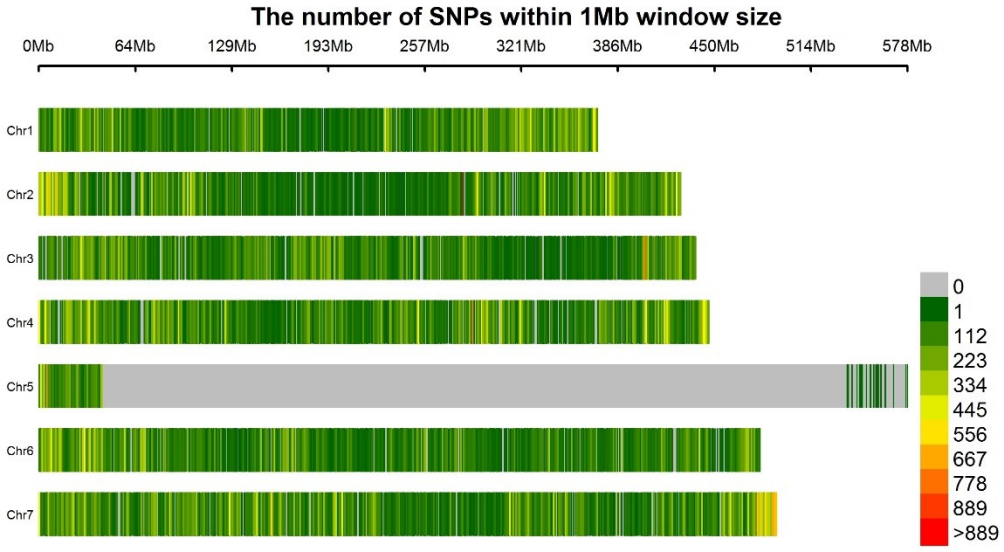


Figure 4.4. Distribution of polymorphic SNPs differing between Aphanomyces root rot-resistant (R) and susceptible (S) pea in three individual R-S pairs and two *in silico* mix bulks on the seven pea chromosomes. The colors indicate SNP density (SNPs/Mb) as per the scale on the right-hand side.

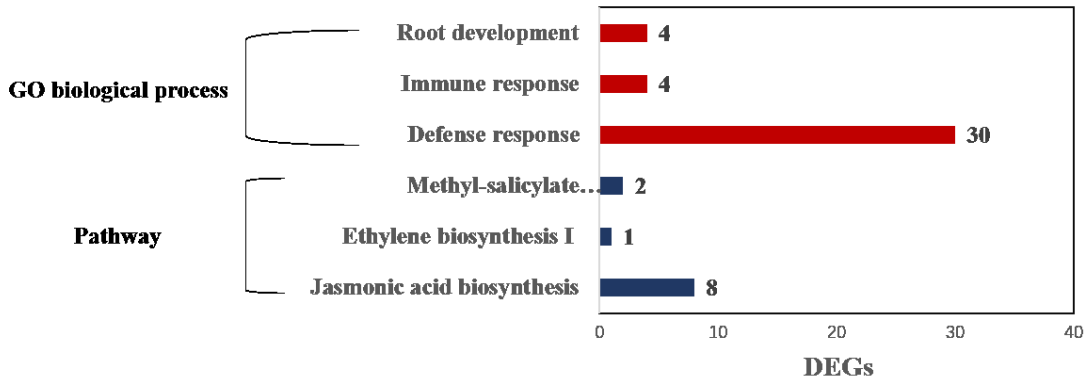


Figure 4.5 Visualization of number of differentially expressed genes in pea resistant or susceptible to Aphanomyces root rot based on GO biological process, including root development (GO:0010015, GO:0010053, GO:0022622 and GO:0048364), immune response (GO:0006955) and defense response (GO:0006952, GO:0031347 and GO:0031348), as well as signaling pathways involving salicylate acid, ethylene and jasmonic acid.

Chapter 5 Summary and general conclusions

5.1 Introduction

The root rot complex is a major limitation to field pea (*Pisum sativum* L.) production in Canada and worldwide. The involvement of multiple pathogens, including *Aphanomyces euteiches* and *Fusarium* spp., increases the difficulties in managing this disease (Pfender et al. 2001; Chang et al. 2005, 2013; Chatterton et al. 2015; Williamson-Benavides et al. 2021). Genetic resistance would represent the most effective and practical tool for root rot control, but no pea cultivars with full resistance are available. In this context, the detection and development of polygenic resistance is a desirable objective for sustainable integrated crop protection. Improved understanding of the genetic control of and mechanisms involved in partial resistance is essential for marker assisted selection, gene pyramiding, and knowledge-based disease management.

This project consisted of three studies to improve understanding of resistance in field pea to *A. euteiches* and *Fusarium* spp. as well as the pea root immune mechanism. In the first study, the basis of resistance to *A. euteiches* in the cultivar '00-02067' was evaluated, with QTL identified using a 13.2K single nucleotide polymorphism (SNP) array in an F_8 RIL population. In the second study, '00-2067' with *Fusarium* spp. commonly associated with the pea root rot complex, and stable QTL associated with partial resistance to *F. graminearum* were identified. In the third and final study, a bulked segregant RNA-seq (BSR-seq) analysis was conducted to characterize *A. euteiches* resistance mechanism(s) in pea.

5.2 Identification of QTL associated with partial resistance to *A. euteiches*

Partial resistance to *A. euteiches* in pea has been shown to be a polygenic and heritable trait (Shehata et al. 1983; Marx et al. 1972; Davis et al. 1995). Several studies using genetic markers have identified major QTL associated with this partial resistance (Pilet-Nayel et al. 2005; Hamon et al. 2011; Lavaud et al. 2015; Desgroux et al. 2016; 2018). The pea cultivar '00-2067', which is the only Canadian source of resistance, was reported to be highly tolerant to *A. euteiches* infection under field conditions in western Canada (Conner et al. 2013). To determine the basis of this

resistance, a 13.2K SNP array and 222 simple sequence repeat (SSR) markers were used to screen an F_8 recombinant inbred line (RIL) population consisting of 135 genotypes derived from the cross '00-2067' × 'Reward'. All phenotypic parameters evaluated had highly significant genotypic effects ($P<0.001$) and G×E interactions ($P<0.05$) in greenhouse and field experiments. Frequency distributions based on the LSMeans of the traits indicated that the data were continuous and tended towards a normal distribution. All traits, except for plant height, were significantly correlated (42-90%) in the greenhouse and field experiments ($P<0.001$). The coincidence between and within field and greenhouse studies enabled fine mapping of the targeted QTL.

A fine genetic map was constructed with the filtered (2978 SNP + 21 SSR) markers and nine linkage groups, which correspond to the seven pea chromosomes. The high marker density contributed to the accuracy of the QTL mapping. Linkage analysis identified 28 QTL, of which 5 had a major-effect (R^2 > 20%). A genomic region on chromosome 4 was identified as the most consistent region responsible for partial resistance to *A. euteiches* isolate Ae-MDCR1. The detected QTL are an important complement to resistance breeding efforts.

5.3 Identification of QTL associated with partial resistance to *Fusarium graminearum*

Quantitative trait loci associated with partial resistance to *F. solani*, *F. avenaceum* and *F. oxysporum* have been reported in field pea (Feng et al. 2010; Mc Phee et al. 2012; Coyne et al. 2015, 2019). To evaluate the broad-spectrum resistance to *Fusarium* spp. in Canadian germplasm, the resistant cultivar '00-2067' and susceptible cultivar 'Reward' were screened with five common *Fusarium* spp., including *Fusarium solani, F. avenaceum, F. acuminatum, F. proliferatum* and *F. graminearum*. The cultivar '00-2067' consistently developed lower root rot severity than 'Reward' following inoculation with *F. avenaceum* or *F. graminearum.* Therefore, fungal isolates representing these two species were selected to screen the F_8 RIL population and identify resistance QTL.

Linkage analysis identified no stable QTL for *F. avenaceum*; in contrast, 8 QTL were detected for *F. graminearum*. The most stable genetic region for partial resistance to *F. graminearum*, related to root rot severity and aboveground vigor, was located on chromosome 4 (LGIV). This region

coincided with the region identified in Chapter 2 for *A. euteiches* resistance with the region reported in Chapter 2, suggesting that it might be important for broad-spectrum resistance to both pathogens.

5.4 BSR-seq analysis for detecting novel genes associated with *A. euteiches* resistance

An increasing number of studies have examined the pea transcriptomes via RNA-seq analysis to improve understanding of root nodulation, seed development and agronomic performance (Alves-Carvalho et al. 2015; Liu et al. 2015; Sudheesh et al. 2015). Bulked segregant RNA-seq analysis, which is more cost-efficient and could detect novel, trait-specific genes, represents another improvement in sequencing technologies. To facilitate marker-assisted selection (MAS), BSR-seq analysis was conducted with an F_8 RIL population derived from the cross 'Carman' × '00-2067'. Novel SNP variants were identified at the gene expression level, which contributed to the development of SNPs that can be used to detect novel QTL controlling partial resistance to *A. euteiches*. Some of the candidate genes appear to be involved in a pathway involving jasmonic acid synthesis. The identification of novel genes associated with partial resistance to *A. euteiches* may facilitate efforts to improve management of this pathogen.

5.5 Future directions

Despite over 50 years of research, breeders and pathologists are still struggling to develop root rot-resistant field pea. The work presented in this thesis identified major and stable QTL associated with partial resistance to *A. euteiches* in the cultivar '00-2067' (Chapter 2); showed the occurrence of resistance QTL effective against *F. graminearum* (Chapter 3); and characterized differentially expressed genes and potential defense pathways involved in pea against *A. euteiches* (Chapter 4). Despite the advances made, however, these results are not on their own sufficient for managing the PRRC. Additional work is needed to improve our knowledge further and to control this disease more effectively.

By taking advantage of the very large numbers of SNP markers designed for pea by Tayeh et al. (2015), we identified QTL in Chapter 2 more precisely than in earlier studies (Pilet-Nayel et al. 2005; Hamon et al. 2011; Lavaud et al. 2015). Nonetheless, the variance explained by our genetic

model was lower than that reported in genomewide association studies by Desgroux et al. (2016, 2018), indicating that of some of the basis for genetic resistance is still waiting to be identified. In addition, given that the SNP array we used was developed for the study of agronomic traits (Tayeh et al. 2015), improved disease-related markers are needed to explore *A. euteiches* resistance further. Since transgressive segregation was observed in Chapter 2, we now also have the possibility of selecting individuals with higher resistance in the RIL populations; these genotypes may be important for breeding programs aimed at controlling ARR.

While broad resistance to *Fusarium* spp. was found in Chapter 3, due to financial constraints only two species were tested for QTL mapping. As a result, stable resistant QTL were identified only for resistance to *F. graminearum*. Greenhouse assessments should be improved for *F. avenaceum*, and additional isolates of other *Fusaria* should be tested. In addition, the resistance to other pathogens implicated in the PRRC, such as *Pythium* spp., *Phytophthora* spp., and *Rhizoctonia solani* J.G. Kühn., should also be characterized in future studies.

Similarly, while we showed the potential of BSR-seq as a tool to investigate the pea/*A. euteiches* interaction in Chapter 4, the detected DEGs and SNPs were not validated. Differential gene expression should be confirmed with techniques such as quantitative PCR, and the potential role(s) of selected genes investigated through strategies such as gene knockouts or yeast two-hybrid assays. The polymorphic SNPs should be confirmed by the Kompetitive Allele Specific PCR (KASP) method and the detected disease-related SNPs added to the former SNP array, helping to detect novel QTL and validate the QTL identified in Chapter 2.

Ultimately, while the genetics of resistance was a major focus of this dissertation, the sustainable management of the PRRC and its associated pathogens will require an integrated approach. For example, seed treatments, risk forecasting, and partially resistant field pea could be combined in the field to achieve effective disease control. In this way, by mitigating the impact of PRRC and other diseases, growers and consumers can continue to enjoy the many benefits of this important crop.

Bibliography

Abbo, S., Rachamim, E., Zehavi, Y., Zezak, I., Lev-Yadun, S., and Gopher, A. (2011). Experimental growing of wild pea in Israel and its bearing on Near Eastern plant domestication. Ann. Bot. 107: 1399-1404.

Afonin, A. M., Leppyanen, I. V., Kulaeva, O. A., Shtark, O. Y., Tikhonovich, I.A., Dolgikh, E.A., and Zhukov, V. A. (2020). A high coverage reference transcriptome assembly of pea (*Pisum sativum* L.) mycorrhizal roots. VavilovskiiZh. Genet. Sel. 24: 331-339. doi: 10.18699/VJ20.625. PMID: 33659815; PMCID: PMC7716550.

Agriculture and Agri-Food Canada (2021) Canada: outlook for principal field crops, 2021-07-20 [Internet]. Ottawa: Minister of Agriculture and Agri-Food. Available from: https://agriculture.canada.ca/en/canadas-agriculture-sectors/crops/reports-and-statistics-data-canadian-principal-field-crops/canada-outlook-principal-field-crops-2021-07-20

Alcalde, J. A., Wheeler, T. R., and Summerfield, R. J. (2000). Genetic characterization of flowering of diverse cultivars of pea. J. Agron. 92: 772-779. https://eurekamag.com/research/003/454/003454641.php

Alves-Carvalho, S., Aubert, G., Carrère, S., Cruaud, C., Brochot, A.-L., Jacquin, F., Klein, A., Martin, C., Boucherot, K., Kreplak, J., da Silva, C., Moreau, S., Gamas, P., Wincker, P., Gouzy, J., and Burstin, J.. (2015). Full length de novo assembly of RNA-seq data in pea (*Pisum sativum* L.) provides a gene expression atlas and gives insights into root nodulation in this species. Plant J. 84:1–19. doi:10.1111/tpj.12967

Anderson, J., Akond, M., Kassem, M. A., Meksem, K., and Kantartzi, S. K. (2015). Quantitative trait loci underlying resistance to sudden death syndrome (SDS) in MD96-5722 by 'Spencer' recombinant inbred line population of soybean. 3 Biotech. 5: 203–210. https://doi.org/10.1007/s13205-014-0211-3.

Ansorge, W. J. (2009). Next-generation DNA sequencing techniques. N Biotechnol. 25: 195–203.

Arabidopsis Genome Initiative (2000). Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature. 408: 796–815.

Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H.L., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S.E., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M., and Sherlock, G. (2000). Gene Ontology: tool for the unification of biology. Nat. Genet. 25:25-29.

Avenot, H., and Michailides, T. (2010). Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. Crop Prot. 29: 643-651.

Badis, Y., Bonhomme, M., Lafitte, C., Huguet, S., Balzergue, S., Dumas, B., and Jacquet, C. (2015). Transcriptome analysis highlights preformed defences and signalling pathways controlled by the prAe1 quantitative trait locus (QTL), conferring partial resistance to *Aphanomyces euteiches* in *Medicago truncatula*. Mol. Plant Pathol. 16: 973-86. doi: 10.1111/mpp.12253.

Bailey, K. L., Gossen, B. D., Gugel, R. K. and Morrall, R. A. A. (2003). Diseases of field crops in Canada. 3rd ed. Canadian Phytopathological Society, University Extension Press, University of Saskatchewan, Saskatoon, SK, Cananda.

Bailey, K. L., Mortensen, I. C., and Lafond, G. P. (1992). Effects of tillage systems and crop rotations on root and foliar diseases of wheat, flax, and peas in Saskatchewan. Can. J. Plant Sci. 22: 583-591.

Bailey, K., and Lazarovits, G. (2003). Suppressing soil-borne diseases with residue management and organic amendments. Soil Tillage Res. 72: 169-180.

Bailey, K., Gossen, B., Derksen, D., and & Watson, P. (2000). Impact of agronomic practices and environment on diseases of wheat and lentil in southeastern Saskatchewan. Can. J. Plant Sci. 80: 917-927.

Bainard, L. D., Navarro-Borrell, A., Hamel, C., Braun, K., Hanson, K., and Gan, Y. (2017). Increasing the frequency of pulses in crop rotations reduces soil fungal diversity and increases the proportion of fungal pathotrophs in a semiarid agroecosystem. Agric. Ecosyst. Environ. 240: 206–214.

Banik, M., Beyene, M., and Wang, X. (2019). Fusarium head blight of barley in Manitoba – 2019. Can. Plant Dis. Surv. 100: 50-51.

Banniza. S., Bhadauria, V., Peluola, C. O., Armstrong-Cho, C., and Morall, R. A. A. (2013). First report of *Aphanomyces euteiches* in Saskatchewan. Can. Plant Dis. Surv. 93: 163– 164.

Basu, P. K., Brown, N. J., Crete, R., Gourley, C. O., Johnston, H. W., Pepin, H. S., and Seaman., W. L. (1976). Yield loss conversion factors for Fusarium root rot of peas. Can. Plant Dis. Surv. 56:5-32.

Bekkering E. (2011). Canadian Agriculture at a Glance: Pulse in Canada. Statistics Canada. Catalogue no. 96‑325‑X, No. 007. ISSN 0-662-35659-4

Bekkering, E. (2013). Pulses in Canada. Statistics Canada. CS96-325/2011-7E-PDF.

Bilgi, V. N., Bradley, C. A., Mathew, F. M., Ali, S., and Rasmussen, J. B. (2011). Root rot of dry edible bean caused by *Fusarium graminearum*. Plant Health Prog. 12. doi: org/10.1094/PHP-2011-0425-01-R

Bing, D. J., Beauchesne, D., Turkington, T. K., Clayton, G., Sloan, A. G., Conner, R. L., Gan, Y., Vera, C., Gelb, D., McLaren, D., Warkentin, T., and Chang, K. F. (2006). Registration of 'Reward' field pea. Crop. Sci. 46: 2705-2706.

Bleau, J. R., and Spoel, S. H. (2021) Selective redox signaling shapes plant–pathogen interactions. Plant Physiol. 186: 53–65, https://doi.org/10.1093/plphys/kiaa088

Bodah, E. T., Porter, L. D., Chaves, B., and Dhingra, A. (2016). Evaluation of pea accessions and commercial cultivars for fusarium root rot resistance. Euphytica. 208: 63–72. doi: 10.1007/s10681-015-1545-6.

Bokore, F. E., Knox, R. E., Cuthbert, R. D., Pozniak, C. J., McCallum, B. D., N'Diaye, A., DePauw, R. M., Campbell, H. L., Munro, C., Singh, A., Hiebert, C. W., McCartney, C. A., Sharpe, A. G., Singh, A. K., Spaner, D., Fowler, D. B., Ruan, Y., Berraies, S., and Meyer, B. (2020). Mapping quantitative trait loci associated with leaf rust resistance in five spring wheat populations using single nucleotide polymorphism markers. PLoS One. 15: e0230855. doi: 10.1371/journal.pone.0230855.

Bokore, F. E., Knox, R. E., Hiebert, C. W., Cuthbert, R. D., DePauw, R. M., Meyer, B., N'Diaye, A., Pozniak, C. J., and McCallum, B. D. (2022). A Combination of Leaf Rust Resistance Genes,

Including Lr34 and Lr46, Is the Key to the Durable Resistance of the Canadian Wheat Cultivar, Carberry. Front. Plant Sci. 12:775383. doi: 10.3389/fpls.2021.775383.

Bolger, M. E., Weisshaar, B., Scholz, U., Stein, N., and Usadel, B. (2014). Mayer FX: Plant genome sequencing–applications for crop improvement. Curr. Opin. Biotechnol. 26:31–37. doi: 10.1016/j.copbio.2013.08.019

Bouchez, O., Huard, C., Lorrain, S., Roby, D., and Balagué, C. (2007). Ethylene is one of the key elements for cell death and defense response control in the Arabidopsis lesion mimic mutant vad1. Plant physiol. 145: 465–477. https://doi.org/10.1104/pp.107.106302

Brautigam, A., Mullick, T., Schliesky, S., and Weber, A.P.M. (2011). Critical assessment of assembly strategies for non-mode lspecies mRNA- Seq data and application of next-generation sequencing to the comparison of C3 and C4 species. J. Exp. Bot. 62: 3093–3102. doi:10.1093/Jxb/Err029

Brim, C. A. (1966). A modified pedigree method of selection in soybean. Crop Sci. 6:220.

Brun, H., Chevre, A. M., Fitt, B. D. L., Powers, S., Besnard, A. L., Ermel, M., Huteau, V., Marquer, B., Eber, and F., Renard, M. (2002). Quantitative resistance increases the durability of qualitative resistance to *Leptosphaeria maculans* in *Brassica napus*. New. Phytol. 185: 285-299.

Brzostowski, L. F., Pruski, T. I., Hartman, G. L., Bond, J. P., Wang, D., Cianzio, S. R., and Diers, B. W. (2018). Field evaluation of three sources of genetic resistance to sudden death syndrome of soybean. Theor. Appl. Genet. 131: 1541-1552. doi: 10.1007/s00122-018-3096-4..

Bueckert, R. A., Wagenhoffer, S. , Hnatowich, G., and Warkentin, T. D. (2015). Effect of heat and precipitation on pea yield and reproductive performance in the field. Can. J. Plant Sci. 95: 629-639. https://doi.org/10.4141/cjps-2014-342

Burke, D. W., and Mitchell, J. E. (1968). Temperature and moisture effects on infection of pea seedlings by *Aphanomyces euteiches* in soil. Phytopathology (Abstract). 58: 1045.

Burke, D. W., Mitchell, J. E., and Hagedorn, D. J. (1969). Selective conditions for infection of pea seedlings by *Aphanomyces euteiches* in soil. Phytopathology 59: 1670-1674.

Burstin, J., Marget, P., Huart, M., Moessner, A., Mangin, B., Duchene, C., Desprez, B., Munier-Jolain, N., and Duc, G. (2007). Developmental Genes Have Pleiotropic Effects on Plant Morphology and

Source Capacity, Eventually Impacting on Seed Protein Content and Productivity in Pea. Plant. Physiol. 144: 768–781

Burstin, J., Salloignon, P., Chabert-Martinello, M., Magnin-Robert, J., Siol, M., Jacquin, F., Chauveau, A., Pont, C., Aubert, G. H., Delaitre, C., Truntzer, C., and Duc, G. (2015). Genetic diversity and trait genomic prediction in a pea diversity panel. BMC Genomics 16: 105 https://doi.org/10.1186/s12864-015-1266-1

Carpenter, M. A., Goulden, D. S., Woods, C. J., Thomson, S. J., Kenel, F., Frew, T. J., Timmerman-Vaughan, G. M. (2018). Genomic Selection for Ascochyta Blight Resistance in Pea. Front. Plant Sci. 9: 1878. doi: 10.3389/fpls.2018.01878

Caspi, R. et al. The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. Nucleic Acids Res. 42, D459–D471 (2014).

Chakraborty, S., Tiedemann, A. V., and Teng, P. S. (2000) Climate change: potential impact on plant diseases. Environ. Pollut. 108: 317-26. doi: 10.1016/s0269-7491(99)00210-9. PMID: 15092926.

Chang K. F., Hwang S. F., Ahmed H. U., Strelkov S. E., Harding M. W., Conner R. L., McLaren D. L., Gossen B. D., and Turnbull G. D. (2018). Disease reaction to *Rhizoctonia solani* and yield losses in soybean. Can. J. Plant Sci. 98: 115–124.

Chang, H. X., Roth, M. G., Wang, D., Cianzio, S. R., Lightfoot, D. A., and Hartman, G. L. (2018). Chilvers MI. Integration of sudden death syndrome resistance loci in the soybean genome. Theor. Appl. Genet. 131: 757-773. doi: 10.1007/s00122-018-3063-0. Epub 2018 Feb 12. PMID: 29435603.

Chang, K. F., Bowness, R., Hwang, S. F., Turnbull, G. D., Howard, R. J., Lopetinsky, K., Olson, M., and Bing, D. J. (2005). Pea diseases occurring in central Alberta in 2004. Can. Plant Dis. Surv. 85: 89-90.

Chang, K. F., Conner, R. L., Hwang, S. F., Ahmed, H. U., McLaren, D. L., Gossen, B. D., and Turnbull, G. D. (2014). Effects of seed treatment and inoculum density of *Fusarium avenaceum* and *Rhizoctonia solani* on seedling blight and root rot of faba bean. Can. J. Plant Sci. 94: 693-700. doi.org/10.4141/cjps2013-339.

Chang, K. F., Hwang, S. F, Ahmed, H., Fu, H., Zhou, Q., Strelkov, S. E. and Turnbull, G. D. (2017).

First report of *Phytophthora sansomeana* causing root rot in field pea in Alberta, Canada. Crop Prot. 101: 1-4. doi: 10.1016/j.cropro.2017.07.008

Chang, K. F., Wu, L. F., Fu, H., Yang, Y., Hwang, S. F., Turnbull, G. D., and Strelkov, S. E. (2016). Microorganisms and the occurrence of root rot of field pea in Alberta in 2015. Can. Plant Dis. Surv. 96: 186-187.

Chatterton, S., Bowness, R., and Harding, M. W. (2015). First Report of Root Rot of Field Pea Caused by Aphanomyces euteiches in Alberta, Canada. Plant Dis. 99: 288. doi: 10.1094/PDIS-09-14-0905-PDN. PMID: 30699595.

Chatterton, S., Harding, M. W., Bowness, R., Mclaren, D. L., Banniza, S., and Gossen, B. D. (2019). Importance and causal agents of root rot on field pea and lentil on the Canadian prairies, 2014–2017, Can. J. Plant Pathol. 41: 98-114. doi: 10.1080/07060661.2018.1547792.

Chen, Y., Zhou, Q., Strelkov, S. E., and Hwang, S. F. (2014). Genetic diversity and aggressiveness of Fusarium spp. isolated from canola in Alberta, Canada. Plant Dis. 98: 727-738.

Chitrampalam, P., and Nelson, B. (2016). Multilocus phylogeny reveals an association of agriculturally important Fusarium solani species complex (FSSC) 11, and clinically important FSSC 5 and FSSC 3 + 4 with soybean roots in the north central United States. Antonie Van. Leeuwenhoek. 109: 335-47. doi: 10.1007/s10482-015-0636-7.

Chittem, K., Mathew, F. M, Gregoire, M., Lamppa, R. S, Chang, Y. W, Markell, S. G, Bradley, C. A, Barasubiye, T., and Goswami, R. S. (2015). Identification and characterization of Fusarium spp. associated with root rots of field pea in North Dakota. Eur. J. Plant. Pathol. 143: 641-649. doi: 10.1007/s10658-015-0714-8

Chongo, G., Gossen, B. D., Kutcher, H. R., Gilbert, J., Turkington, T. K., Femandez, M. R., and Mclaren, D. (2001). Reaction of seedling roots of 14 crop species to *Fusarium graminearum* from wheat heads. Can. J. Plant Pathol. 23: 132-137.

Chu, C., Wang, S., Paetzold, L., Wang, Z., Hui, K., Rudd, J.C., Xue, Q., Ibrahim, A.M., Metz, R.P., Johnson, C.D., Rush, C.M., & Liu, S. (2021). RNA-seq analysis reveals different drought tolerance mechanisms in two broadly adapted wheat cultivars ‘TAM 111’ and ‘TAM 112’. Sci Rep 11: 4301.

https://doi.org/10.1038/s41598-021-83372-0

Cichy, K. A., Snapp, S., and Kirk, W. W. (2007), Fusarium root rot incidence and root system architecture in grafted common bean lines. Plant Soil. 300: 233-244. doi: 10.1007/s11104-007-9408-0.

Clarkson, J. D. S. (1978). Pathogenicity of Fusarium spp. associated with foot-rots of peas and beans. Pl. Path. 27: 110-117

Conner, R. L., Chang, K. F., Hwang, S. F., Warkentin, T. D. and McRae, K. B. (2013). Assessment of tolerance for reducing yield losses in field pea caused by *Aphanomyces* root rot. Can. J. Plant Sci. 93: 473-482.

Conner, R. L., Hou, A., Balasubramanian, P., McLaren, D. L., Henriquez, M. A., Chang, K.-F., and McRaeKenneth B. (2014). Reaction of dry bean cultivars grown in western Canada to root rot inoculation. Can. J. Plant Sci. 94: 1219–1230. doi: 10.4141/ cjps2013-416.

Coyne, C. J., Porter, L. D., Boutet, G. Ma, Y., McGee, R. J., Lesne, A., Baranger, A., and Pilet-Nayel, M. (2019). Confirmation of Fusarium root rot resistance QTL Fsp-Ps 2.1 of pea under controlled conditions. BMC Plant Biol. 19: 98. doi: 10.1186/s12870-019-1699-9

Coyne, C., Pilet-Nayel, M., Mcgee, R., Porter, L., Smýkal, P., and Grünwald, N. (2015). Identification of QTL controlling high levels of partial resistance to *Fusarium solani* f. sp. *pisi* in pea. Plant Breed. doi: 10.1111/pbr.12287

Das, P., Nutan, K. K., Singla-Pareek, S. L., and Pareek, A. (2015). Oxidative environment and redox homeostasis in plants: dissecting out significant contribution of major cellular organelles. Frontiers in Environmental Science, 2. doi:10.3389/fenvs.2014.00070

Davidson, J. A., Hartley, D., Priest, M., Herdina, M. K. K., McKay, A., and Scott, E. S. (2009). A new species of Phoma causes ascochyta blight symptoms on field peas (*Pisum sativum*) in South Australia. Mycologia. 101: 120–128. doi: 10.3852/07-199

Davis, D. W., Fritz, V. A., Pfleger, 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. Hort. Sci. 30: 639–640

Delwiche, P. A., Grau, C. R., Holub, E. B., and Perry, J. B. (1987). Characterization of *Aphanomyces euteiches* isolates recovered from alfalfa in Wisconsin. Plant Dis. 71: 155-161.

Desgroux A, L'Anthoëne V, Roux-Duparque M, Rivière J, Aubert G, Tayeh N, Pilet-Nayel M (2016) Genome-wide association mapping of partial resistance to *Aphanomyces euteiches* in pea. BMC Genom 17:124. doi:10.1186/s12864-016-2429-4

Desgroux, A., Baudais, V. N., Aubert, V., Le Roy, G., de Larambergue, H., Miteul, H., Aubert, G., Boutet, G., Duc, G., Baranger, A., Burstin, J., Manzanares-Dauleux, M., Pilet-Nayel, M-L., and Bourion, V. (2018). Comparative Genome-Wide-Association Mapping Identifies Common Loci Controlling Root System Architecture and Resistance to Aphanomyces euteiches in Pea. Front. Plant Sci. 8:2195. doi: 10.3389/fpls.2017.02195.

Díaz Arias, M. M., Munkvold G. P., and Leandro L. F. (2011). First Report of *Fusarium proliferatum* Causing Root Rot on Soybean (Glycine max) in the United States. Plant Dis. 95: 1316. doi: 10.1094/PDIS-04-11-0346. PMID: 30731665.

Dorrance, A. E., McClure, S. A., and St Martin, S. K. (2003). Effect of partial resistance on Phytophthora stem rot incidence and yield of soybean in Ohio. Plant Dis. 87: 308–312.

Duarte, J., Rivière, N., Baranger, A., Aubert, G., Burstin, J., Cornet, L., Lavaud, C., Lejeune-Hénaut, I., Martinant, J. P., Pichon, J. P., Pilet-Nayel, M. L., Boutet, G.. (2014). Transcriptome sequencing for high throughput SNP development and genetic mapping in pea. BMC Genomics. 15:126.

Elad, Y., and Pertot, I. (2014). Climate Change Impacts on Plant Pathogens and Plant Diseases, J. Crop Improv. 28: 99-139, doi: 10.1080/15427528.2014.865412

Endres, G., Forster, S., Kandel, H., Pasche, J., Wunsch, M., Knodel, J., and Hellevang, K. (2016). Field pea production. North Dakota State University. A1166. https://www.ag.ndsu.edu/publications/crops/field-pea-production.

Eskridge, K. M. (1995). Statistical analysis of disease reaction data using nonparametric methods. Hort. Sci. 30: 478—481.

Feng, J., Hwang, R., Chang, K. F., Conner, R. L., Hwang, S. F., Strelkov, S. E., Gossen, B. D., McLaren, D. L., and Xue, A. G. (2011). Identification of microsatellite markers linked to

quantitative trait loci controlling resistance to Fusarium root rot in field pea. Can. J. Plant Sci. 91: 199-204.

Feng, J., Hwang, R., Chang, K. F., Hwang, S. F., Strelkov, S.E., Gossen, B.D., Conner, R.L. and Turnbull, G.D. (2010). Genetic variation in *Fusarium avenaceum* causing root rot on field pea. Plant Pathol. 59: 413-421.

Fernandez, M. R. (2007). Fusarium populations in roots of oilseed and pulse crops grown in eastern Saskatchewan. Can. J. Plant Sci. 87: 945–952.

Fernandez, M. R., Huber, D., Basnyat, P., and Zentner, R. P. (2008). Impact of agronomic practices on populations of Fusarium and other fungi in cereal and noncereal crop residues on the Canadian prairies. Soil Tillage Res. 100: 60–71.

Fletcher, J., Broadhurst, P., and Bansal, R. K. (1991). *Fusarium avenaceum*: A pathogen of lentil in New Zealand. N. Z. J. Crop Hortic. 19: 207-210.

Foroud, N. A., Chatterton, S., Reid, L. M., Turkington, T. K., Tittlemier, S. A., and Gr¨afenhan, T. (2014). Fusarium diseases of Canadian grain crops: Impact and disease management strategies. Pages 267-316 in: Future Challenges in Crop Protection Against Fungal Pathogens. A. Goyal and C. Manoharachary, eds. Springer, New York.

Franssen, S. U., Shrestha, R. P., Bräutigam, A., Bornberg-Bauer, E., and Weber, A. P. M. (2011). Comprehensive transcriptome analysis of the highly complex Pisum sativum genome using next generation sequencing. BMC Genomics. 12: 227.

Frederickson Matika, D. E., and Loake, G. J. (2014). Redox regulation in plant immune function. Antioxidants & redox signaling, 21: 1373–1388. https://doi.org/10.1089/ars.2013.5679

Fredua-Agyeman, R., Jiang, J., Hwang, S-F., and Strelkov, S. E. (2020). QTL Mapping and Inheritance of Clubroot Resistance Genes Derived from Brassica rapa subsp. rapifera ECD 02 Reveals Resistance Loci and Distorted Segregation Ratios in Two F2 Populations of Different Crosses. Front. Plant Sci. 11:899. doi: 10.3389/fpls.2020.00899.

Gali, K. K., Yong, L., Anoop, S., Marwan, D., Arun, S. K., Gene, A., Ketema, D., Carolyn, C., Reddy, L., Bunyamin, T., and Thomas, D. W. (2018). Construction of high-density linkage maps for

mapping quantitative trait loci for multiple traits in field pea (*Pisum sativum* L.). BMC Plant Biol. 18: 172. doi: 10.1186/s12870-018-1368-4

Garg, R., and Jain, M. (2013). RNA-Seq for Transcriptome Analysis in Non-model Plants. In: Rose R. (eds) Legume Genomics. Methods in Molecular Biology (Methods and Protocols), vol 1069. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-62703-613-9_4

Gautam, H. R., Bhardwaj, M., and Kumar, R. (2013). Climate change and its impact on plant diseases. Current Science 105: 1685-1691.

Gill, K. (2018). Crop rotations compared to continuous canola and wheat for crop production and fertilizer use over six years. Can. J. Plant Sci. 98. doi: 10.1139/CJPS-2017-0292.

Goff, S. A., Schnable, J. C., and Feldmann, K. A. (2014). The evolution of plant gene and genome sequencing In Paterson A. [ed.], Genomes of herbaceous land plants, 47–90. Academic Press, Cambridge, Massachusetts, USA.

Gordon, W. L. (1944). The occurrence of Fusarium species in Canada. I. Species of Fusarium isolated from farm samples of cereal seed in Manitoba. Can. J. Res. 22: 282-286.

Gossen, B. D., Conner, R., Henriquez, M., Chang, K. F., Hwang, S. F., Pasche, J., and Chatterton, S. (2016). Identifying and managing root rot of pulses on the northern Great Plains. Plant Dis. 100: 1965-1978.

Goswami, R. S., Dong, Y., and Punja, Z. K. (2008). Host range and mycotoxin production by *Fusarium equiseti* isolates originating from ginseng fields1, Can. J. Plant Pathol. 30: 155-160. doi: 10.1080/07060660809507506

Govaerts B., Mezzalama M., Unno Y., Sayre K. D., Luna-Guido M., Vanherck K., Dendooven L., and Deckers J. (2007). Influence of tillage, residue management, and crop rotation on soil microbial biomass and catabolic diversity. Appl. Soil Ecol. 37: 18-30

Gregoire, M., and Bradley, C. (2005). Survey of root rot diseases affecting dry pea in North Dakota. (Abstr.). Phytopathology 95: S36.

Gritton, E. T. (1990). Registration of 5 root-rot resistant germplasm lines of processing pea. Crop Sci. 30: 1166-1167.

Grünwald, N. J., Coffman, V. A., and Kraft, J. M. (2003). Sources of Partial Resistance to Fusarium Root Rot in the Pisum Core Collection. Plant Dis. 87:1197-1200. doi: 10.1094/PDIS.2003.87.10.1197.

Gritton, E. T. (1995). Offer of seed from the Earl Gritton pea improvement program at Madison. Pisum Genet. 27: 29-30

Gu, Y., Zavaliev, R., and Dong, X. (2017). Membrane Trafficking in Plant Immunity. Mol. Plant. 10: 1026–1034. https://doi.org/10.1016/j.molp.2017.07.001

Gunawardana, D., Likic, V., and Gayler, K. R. (2009). A comprehensive bioinformatics analysis of the Nudix superfamily in Arabidopsis thaliana. Comp. Funct. Genomics 2009:820381.

Haenseler, C. M. (1926). Studies on the root rot of peas (*Pisum sativum*) caused by *Aphanomyces euteiches* Drechsler. New Jersey Agricultural Experiment Station 46th Annual Report. 46: 467-484.

Hall, R., Schwartz, H. F., Steadman, J. S., and Forster, R. (2005). Compendium of bean disease. Seconded. American Phytopathological Society Press, St. Paul, Mn.

Hagedorn, D. J. (1984). Anthracnose. In: D.J. Hagedorn (ed-), Compendium of Pea Diseases. The American Phytopathological Society. pp. 15

Hamon, C., Baranger, A., Coyne, C. J., McGee, R. J., Le Goff, I., L'Anthoene, V., and Lesne, A. (2011). New consistent QTL in pea associated with partial resistance to *Aphanomyces euteiches* in multiple French and American environments. Theo. Appl. Genet. 123: 261-281.

Hamon, C., Coyne, C. J., McGee, R. J., Lesné, A., Esnault, R., Mangin, P., and Pilet-Nayel, M. (2013). QTL meta-analysis provides a comprehensive view of loci controlling partial resistance to *Aphanomyces euteiches* in four sources of resistance in pea. BMC Plant. Biol. 13: 1-19.

Henry, L., and Wickham, H. (2020). purrr: Functional Programming Tools. R package version 0.3.4. https://CRAN.R-project.org/package=purrr

Holliday, P. (1980). Fungal diseases of tropical crops. Cambridge University Press, Cambridge, UK. p. 2527.

Hospital, F. (2009). Challenges for effective marker-assisted selection in plants. Genetica 136: 303–

310

Hossain, S., Bergkvist, G., Berglund, K., Mårtensson, A., and Persson, P. (2012). Aphanomyces pea root rot disease and control with special reference to impact of Brassicaceae cover crops. Acta Agric. Scand.: Section B, Soil Plant Sci. 62: 477-487.

Hossain, S., Bergkvist, G., Glinwood, R. Berglund, K., Martensson, A., Hallin, S., Persson, P. (2015). Brassicaceae cover crops reduce Aphanomyces pea root rot without suppressing genetic potential of microbial nitrogen cycling. Plant Soil. 392: 227–238. https://doi.org/10.1007/s11104-015-2456-y.

Hosseini, S.S., Elfstrand, M., Heyman, F., Funck Jensen, D., and Karlsson, M. (2015). Deciphering common and specific transcriptional immune responses in pea towards the oomycete pathogens *Aphanomyces euteiches* and *Phytophthora pisi*. BMC Genomics. 16: 627. https://doi.org/10.1186/s12864-015-1829-1.

Hu, J., Li, J., Wu, P., Li, Y., Qiu, D., Qu, Y., Xie, J., Zhang, H., Yang, L., Fu, T., Yu, Y., Li, M., Liu, H., Zhu, T., Zhou, Y., Liu, Z., and Li, H. (2019). Development of SNP, KASP, and SSR Markers by BSR-Seq Technology for Saturation of Genetic Linkage Map and Efficient Detection of Wheat Powdery Mildew Resistance Gene Pm61. Int. J. Mol. Sci. 20:750. doi: 10.3390/ijms20030750.

Hwang, S. F., and Chang, K. F. (1989). Incidence and severity of root rot disease complex of field pea in northeastern Alberta in 1988. Can. Plant Dis. Surv. 69: 139-141

Hwang, S. F., Gossen, B. D., Turnbull, G. D., Chang, K. F., Howard, R. J., and Thomas, A. G. (2000). Effect of temperature, seeding date, fungicide seed treatment and inoculation with *Fusarium avenaceum* on seedling survival, root rot severity and yield of lentil. Can. J. Plant. Sci. 80: 899-907

Hwang, S. F., Howard, R. J., Chang, K. F., Park, B., and Burnett, P. A. (1994). Etiology and severity of Fusarium root rot of lentil in Alberta. Can. J. Plant Pathol. 16: 295-303.

International Rice Genome Sequencing Project (2005). The map-based sequence of the rice genome. Nature. 436: 793–800.

International wheat genome sequencing consortium (IWGSC). (2018). Shifting the limits in wheat

research and breeding using a fully annotated reference genome. Science. doi: https://doi.org/10.1126/science.aar7191.

Iquebal, M.A., Sharma, P., Jasrotia, R.S., Jaiswal, S., Kaur, A., Saroha, M., Angadi, U.B., Sheoran, S., Singh, R., Singh, G.P., Rai, A., Tiwari, R., & Kumar, D. (2019). RNAseq analysis reveals drought-responsive molecular pathways with candidate genes and putative molecular markers in root tissue of wheat. Sci. Rep. 9: 13917 https://doi.org/10.1038/s41598-019-49915-2

Jacquet, C., and Bonhomme, M. (2019). Deciphering resistance mechanisms to the root rot disease of legumes caused by *Aphanomyces euteiches* with Medicago truncatula genetic and genomic resources. In: The Model Legume *Medicago truncatula*, F. de Bruijn (Ed.). https://doi.org/10.1002/9781119409144.ch37

Jewell, J. B., Sowders, J. M., He, R., Willis, M. A., Gang, D. R., and Tanaka, K. (2019) Extracellular ATP Shapes a Defense-Related Transcriptome Both Independently and along with Other Defense Signaling Pathways. Plant Physiol. 179:1144-1158. doi: 10.1104/pp.18.01301.

Jinks, J. L., and Pooni, H. S. (1976). Predicting the properties of recombinant inbred lines derived by single seed descent. Heredity 36: 253-266.

Johansen, C., Krishnamurthy, L., Saxena, N. P., Sethi, S. C., (1994). Genotypic variation in moisture response of chickpea grown under line-source sprinklers in a semi-arid tropical environment. Field Crops Res. 37: 103–112.

Jone, F. R., and Drechsler, C. (1925). Root rot of peas in the United States caused by *Aphanomyces euteiches*. J. Agric. Res. 30: 293-325.

Jones, J. D., Dangl, J. L. (2006). The plant immune system. Nature. 444:323–329.

Kahvejian, A., Quackenbush, J., and Thompson, J. F. (2008). What would you do if you could sequence everything? Nat Biotechnol. 26: 1125–1133.

Kaiser, W. J. (1992). Fungi associated with the seeds of commercial lentils from the US Pacific Northwest. Plant Dis. 76: 605–610.

Kaiser, W. J. (1992). Fungi associated with the seeds of commercial lentils from the US Pacific Northwest. Plant Dis. 76 : 605–610.

Kanehisa, M. and Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 28, 27–30.

Karim, M. M., Dakouri, A., Zhang, Y., Chen, Q., Peng, G., Strelkov, S. E., Gossen, B.D., and Yu, F. (2020). Two Clubroot-Resistance Genes, Rcr3 and Rcr9wa, Mapped in Brassica rapa Using Bulk Segregant RNA Sequencing. Int. J. Mol. Sci. 21: 5033. https://doi.org/10.3390/ijms21145033.

Kaur, S., Pembleton, L., Cogan, N. O. I., Savin, K. W., Leonforte, T., Paull, J., Materne, M., and Forster, J. W. 2012. Transcriptome sequencing of field pea and faba bean for discovery and validation of SSR genetic markers. BMC Genomics. 13:104.

Kerr, A. (1962). The root rot-Fusarium wilt complex of pea. Aust. J. Biol. Sci. 16: 55—69.

King, T. H., Johnson, H. G., Bissonnettee, H., and Haglund, W. A. (1960). Development of lines of Pisum sativum resistant to Fusarium root rot and wilt. Proc. Am. Hort. Sci. 75: 510-516.

Klimek-Kopyra, A., Zając, T., Skowera, B., and Styrc, N. (2017). The effect of water shortage on pea (*Pisum sativum* L.) productivity in relation to the pod position on the stem. Acta Agrobot. 70: 1719. https://doi. org/10.5586/aa.1719.

Knott, C. M. (1987). A key for stages of development of the pea (*Pisum sativum*). Ann. Appl. Biol., 111: 233-245.

Kosambi, D. D. (1944) The Estimation of Map Distances from Recombination Values. Ann. Eugen. 12: 172-175.

Kosgey, Z. C., Edae, E. A., Dill-Macky, R., Jin, Y., Bulbula, W. D., Gemechu, A., Macharia, G., Bhavani, S., Randhawa, M. S., and Rouse, M. N. (2021). Mapping and Validation of Stem Rust Resistance Loci in Spring Wheat Line CI 14275. Front. Plant Sci. 11:609659. doi: 10.3389/fpls.2020.609659.

Kou, Y. J., and Wang, S. P. (2010). Broad-spectrum and durability: understanding of quantitative disease resistance. Curr. Opin. Plant Biol. 13: 181-185.

Kraft, J. M. (1974). The Influence of Seedling Exudates on the Resistance of Peas to Fusarium and Pythium Root Rot. Phytopathology 64: 190-193.

Kraft, J. M. (1984). Fusarium Root Rot. In: Hagedorn D.J. (Ed.), Compendium of Pea Diseases.

Am. Phytopathol. Soc., pp: 30-31.

Kraft, J. M. (1992). Registration of 90-2079, 90-2131 and 90-2322 pea germplasms. Crop Sci. 32: 1076-1076.

Kraft, J. M., and Burke, D. W. (1974). Behaviour of *Fusarium solani* f. sp.pisi and *Fusarium solani* f.sp.phaseoli individually and in combinations on peas and beans. Plant Dis. Reporter 58: 500–504.

Kraft, J. M., and Coffman, V. A. (2000a). Registration of 97-261 and 97-2154 Pea Germplasms. Crop Sci. 40: 302. 10.2135/cropsci2000.0007rgp.

Kraft, J. M., and Coffman, V. A. (2000b). Registration of 97-363, 97-2170, and 97-2162 Pea Germplasms. Crop Sci. 40: 303. 10.2135/cropsci2000.0008rgp.

Kraft, J. M., and Pfleger, F. L. (2001). Compendium of Pea Diseases and Pests (2nd ed.). St. Paul: The American Phytopathological Society.Kraft, J.M., (1981). Registration of 792022 and 792024 pea germplasm (Reg Nos. GP21 and GP22). Crop Sci. 21: 352-353.

Kraft, J. M., Dunne, B., Goulden, D., and Armstrong, S. (1998). A search for resistance in peas to Mycosphaerella pinodes. Plant Dis. 82: 251–253.

Kreplak. J., Madoui, M. A., Cápal, 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., Lichtenzveig, 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. https://doi.org/10.1038/s41588-019-0480-1

Kuczyn′ ska, A., Surma, M., and Adamski, T. (2007). Methods to predict transgressive segregation in barley and other selfpollinated crops. J. Appl. Genet. 48: 321-328.

Kumar, R.R., Goswami, S., Sharma, S.K., Kala, Y.K., Rai, G.K., Mishra, D.C., Grover, M., Singh, G., Pathak, H., Rai, A., Chinnusamy, V., and Rai, R.D. (2015). Harnessing Next Generation Sequencing in Climate Change: RNA-Seq Analysis of Heat Stress-Responsive Genes in Wheat

(Triticum aestivum L.). Omics : a journal of integrative biology, 19: 632-47.

Labrousse, F. (1933). Notes de pathologievégétale. Revue de pathologievégétale et d'Entomologieagricole . 19: 71–84.

Lang. D. T. (2022). RCurl: General Network (HTTP/FTP/...) Client Interface for R. R package version 1.98-1.6. https://CRAN.R-project.org/package=RCurl

Lavaud, C., Lesné, A., Piriou, C., Roy, G., Boutet, G., Moussart, A., and Pilet-Nayel, M. (2015). Validation of QTL for resistance to *Aphanomyces euteiches* in different pea genetic backgrounds using near-isogenic lines. Theor. Appl. Genet. 128: 2273-2288.

Lawrence, M., Huber, W., Pagès, H., Aboyoun, P., Carlson, M., Gentleman, R., Morgan, M. T., Carey, V. J. (2013). Software for Computing and Annotating Genomic Ranges. PLoS Comput. Biol. 9: e1003118. doi:10.1371/journal.pcbi.1003118

Lazányi, J. (2002). Trends in Dry Pea (*Pisum sativum* L.) Production. Acta Agraria Debreceniensis. 53–58. https://doi.org/10.34101/actaagrar/1/3537.

Levitt, J., (1980). Response of plants to environmental stresses. Academic Press. New York.

Li, M., Gordon, M., and Gavin, A. (2007). New root diseases of canola in Australia. Australas. Plant Dis. Notes. 2: 93-94. doi:10.1071/DN07038.

Li, W. J., Feng, J., Chang, K. F., Conner, R. L., Hwang, S. F., Strelkov, S. E., Gossen, B. D., and McLaren, D. L. (2012). Microsatellite DNA Markers Indicate Quantitative Trait Loci Controlling Resistance to Pea Root Rot Caused by *Fusarium avenaceum* (Corda ex Fries) Sacc. Plant Pathol. J. 11: 114-119.

Liao, Y., Smyth, G. K., and Shi, W. (2019). The R package Rsubread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. Nucleic Acids Res. 47: e47.

Lincoln, S. M., Daly, M. J., and Lander, E. S. (1992). Constructing genetic maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report.

Liu, J. F., Cao, T. S., Feng, J., Chang, K. F., Hwang, S. F., and Strelkov, S. E. (2013). Characterization of the fungi associated with ascochyta blight of field pea in Alberta, Canada. Crop Prot. 54: 55–64. doi: 10.1016/j.cropro.2013.07.016

Liu, N., Zhang, G., Xu, S., Mao, W., Hu, Q., and Gong, Y. (2015). Comparative transcriptomic analyses of vegetable and grain pea (*Pisum sativum* L.) seed development. Front. Plant Sci. 6:1039. doi: 10.3389/fpls.2015.01039.

Liu, S., Yeh, C.T., Tang, H. M., Nettleton, D., and Schnable, P. S. (2012). Gene Mapping via Bulked Segregant RNA-Seq (BSR-Seq). PLOS ONE 7: e36406. https://doi.org/10.1371/journal.pone.0036406

Liu, X., Hu, X., Li, K. et al. (2020). Genetic mapping and genomic selection for maize stalk strength. BMC Plant Biol. 20: 196. https: //doi.org/10.1186/s12870-020-2270-4

Liu, X., Wang, H., Hu, X., Li, K., Liu, Z., Wu, Y., and Huang, C. (2019). Improving Genomic Selection with Quantitative Trait Loci and Nonadditive Effects Revealed by Empirical Evidence in Maize. Front. Plant Sci. 10: 1129. doi: 10.3389/fpls.2019.01129. PMID: 31620155; PMCID: PMC6759780.

Lockwood, J. L. and Ballard, J. C. (1960). Evaluation of pea introductions for resistance to Aphanomyces and Fusarium root rots. Michigan Quarterly Bulletin 42: 704-713.

Loridon, K., Mc Phee, K., Morin, J., Dubreuil, P., Pilet-Nayel, M. L., Aubert, G., Rameau, C., Baranger, A., Coyne, C., Lejeune-He`naut, I., and Burstin, J. (2005). Microsatellite marker polymorphism and mapping in pea (Pisum sativum L.). Theor. App. Genet. 111: 1022-1031.

Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology. 15:550. https://doi.org/10.1186/s13059-014-0550-8

Lubberstedt, T., Melchinger, A. E., Schon, C. C., Utz, H., and Klein, D. (1997). QTL mapping in testcrosses of European flint lines of maize. I. Comparison of different testers for forage yield traits. Crop Sci. 37: 921–931.

Macas, J., Neumann, P., and Navrátilová, A. (2007). Repetitive DNA in the pea (*Pisum sativum* L.) genome: comprehensive characterization using sequencing and comparison to soybean and *Medicago truncatula*. BMC Genom. 8: 427. doi: 10.1186/1471-2164-8-427

Malovichko, Y. V., Shtark, O. Y., Vasileva, E. N., Nizhnikov, A. A., and Antonets, K. S. (2020).

Transcriptomic Insights into Mechanisms of Early Seed Maturation in the Garden Pea (*Pisum sativum* L.). Cells. 9: 779. https://doi.org/10.3390/cells9030779

Malvick, D. K., Percich, J. A., Pfleger, F. L., Givens, J., and Williams, J. L. (1994). Evaluation of methods for estimating inoculum potential of *Aphanomyces euteiches* in soil. Plant Dis. 78: 361-365.

Manning, M., and Menzies, S. A. (1984). Pathogenic variability in isolates of Aphanomyces euteiches from New Zealand soils. New Zeal. J. Agr. Res. 27: 569-574.

Mathew, F. M., Lamppa, R. S., Chittem, K., Chang, Y. W., Botschner, M., Kinzer, K., Goswami, R. S., and Markell, S. G. (2012). Characterization and pathogenicity of Rhizoctonia solani isolates affecting Pisum sativum in North Dakota. Plant Dis. 96: 666–672.

Marguerat, S., Wilhelm, B. T., and Bähler, J. (2008). Next-generation sequencing: applications beyond genomes. Biochem. Soc. Trans. 36:1091–6.

Marx, G. A., Schroeder, W. T., Provvidenti, R., and Mishanee, W. (1972). A genetic study of tolerance in pea (*Pisum sativum* L.) to Aphanomyces root rot. J. Am. Hortic. Sci. 97: 619621.

Matuo, T., Snyder, W. C. (1973). Use of morphology and mating populations in the identification of formaespeciales in Fusarium solani. Phytopathology. 63: 562–565.

McLaren, D. L., Henderson, T. L., Kim, Y. M., Chang, K. F., and Kerley, T.J. (2016). Field pea disease in Manitoba in 2015. Can. Plant Dis. Surv. 96:191-192.

Mc Phee, K. E., Inglis, D. A., Gundersen, B., and Coyne, C. J. (2012). Mapping QTL for Fusarium wilt Race 2 partial resistance in pea (*Pisum sativum*). Plant Breed. 131: 300–306. doi:10.1111/j.1439-0523.2011. 01938.x

McKay, K., Schatz, B. G., and Endres, G. (2003). Field pea production. NDSU Extension Service.

McPhee, K. (2005). Variation for seedling root architecture in the core collection of pea germplasm. Crop Sci. 45: 1758–1763. doi: 10.2135/cropsci2004.0544.

Medicago Truncatula Genome Project. (2015). [http://jcvi.org/medicago/]

Meng, L., Li, H., Zhang, L., and Wang, J. (2015). QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in bi-parental populations. Crop J.

3: 269-283

Modderman, C. T., Markell, M., Wunsch, M., and Pasche J. S. (2018). Efficacy of In-Furrow Fungicides for Management of Field Pea Root Rot Caused by Fusarium avenaceum and *F. solani* Under Greenhouse and Field Conditions. Plant Health Progress. 19: 212-219.

Naithani, S., Preece, J., D'Eustachio, P., Gupta, P., Amarasinghe, V., Dharmawardhana, P., Wu, G., Fabregat, A., Elser, J., Weiser, J., Keays, M., Fuentes, A.M., Petryszak, R., Stein, L., Ware, D.H., and Jaiswal, P. (2017). Plant Reactome: a resource for plant pathways and comparative analysis. Nucleic Acids Res. 45: D1029-D1039.

Nakedde, T., Ibarra-Perez, F. J., Mukankusi, C., Waines, J. G., and Kelly, J. D. (2016). Mapping of QTL associated with Fusarium root rot resistance and root architecture traits in black beans. Euphytica. 212: 51-63

Navarro, F., Sass, M. E., and Nienhuis, J. (2008). Identification and confirmation of quantitative trait loci for root rot resistance in snap bean. Crop Sci. 48: 962-972

Neumann, P., Pozárková, D., Vrána, J., Dolezel, J., and Macas, J. (2002). Chromosome sorting and PCR-based physical mapping in pea (*Pisum sativum* L.). Chromosome Res. 10: 63-71. doi: 10.1023/a:1014274328269.

Nguyen NT, Lindsey ML, Jin YF. Systems analysis of gene ontology and biological pathways involved in post-myocardial infarction responses. BMC Genomics. 2015;16 Suppl 7(Suppl 7):S18. doi: 10.1186/1471-2164-16-S7-S18. Epub 2015 Jun 11. PMID: 26100218; PMCID: PMC4474415.

O'Donnell, K., (2000). Molecular phylogeny of the Nectriahaematococca- *Fusarium solani* species complex. Mycologia. 92: 919–938.

Obenchain, V., Lawrence, M., Carey, V., Gogarten, S., Shannon, P., and Morgan, M. (2014). "VariantAnnotation: a Bioconductor package for exploration and annotation of genetic variants." Bioinformatics 30: 2076-2078. doi: 10.1093/bioinformatics/btu168.

Okada, K., Abe, H., Arimura, G. (2015). Jasmonates induce both defense responses and communication in monocotyledonous and dicotyledonous plants. Plant Cell Physiol. 56:16-27. doi: 10.1093/pcp/pcu158.

Okubara, P.A., and Paulitz, T.C. (2005). Root Defense Responses to Fungal Pathogens: A Molecular Perspective. Plant Soil 274: 215–226. https://doi.org/10.1007/s11104-004-7328-9

Olson, B., Blois, T., Ernst, B., Japp, M., Junek, S., Kutcher, H.R., Prasd, T., and Ziesman, B. (2019). Seed-borne fusarium on cereal crops in Saskatchewan in 2018. Can. Plant Dis. Surv. 100: 100-103.

Oyarzun, P. J. (1993). Bioassay to assess root rot in pea and effect of root rot on yield. Neth. J. Pl. Path. 99: 61-75

Oyarzun, P., Gerlagh, M., Hoogland, A., and Vos, I. (1990). Seed treatment of peas with fosetyl-Al against *Aphanomyces euteiches*. Nether. J. Plant Pathol. 96: 301-311.

Oyarzun, P., Gerlagh, M., Hoogland, A., and Vos, I. (1990). Seed treatment of peas with fosetyl-Al against *Aphanomyces euteiches*. Nether. J. Plant Pathol. 96: 301-311.

Palloix, A., Ayme, V., and Moury, B. (2009). Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. New Phytol. 183: 190-199.

Pande, A., and Rao, V. G. (1998). A compendium Fungi on Legumes from India. Scientific Publishers, Jodhpur, India.

Papavizas, G., and Ayers, W. (1974). *Aphanomyces* species and their root diseases on pea and sugarbeet. US Dept. Agric. Agric. Res. Serv. Tech. Bull 1485: 158.

Paradis, E. (2010). pegas: an R package for population genetics with an integrated-modular approach. Bioinformatics 26: 419-420.

Perez-Lara, E., Semagn, K., Chen, H., Iqbal, M., N'Diaye, A., Kamran, A., Navabi, A., Pozniak, C., and Spaner, D. (2016). QTLs Associated with Agronomic Traits in the Cutler × AC Barrie Spring Wheat Mapping Population Using Single Nucleotide Polymorphic Markers. PLOS ONE 11: e0160623. https: //doi.org/10.1371/journal.pone.0160623

Persson, L., Bødker, L., and Larsson-Wikström, M. (1997). Prevalence and pathogenicity of foot and root rot pathogens of pea in southern Scandinavia. Plant Dis. 81: 171–174.

Pfender, W. F., and Hagedorn, D. J. (1983). Disease progress and yield loss in Aphanomyces root rot of peas. Phytopathol. 73: 1109-1113.

Pfender, W. F., Delwiche, P. A., Grau, C. R., and Hagedorn, D. J. (1984). A medium to enhance recovery of Aphanomyces from infected plant tissue. Plant Dis. 68: 845-847.

Pfender, W., Malvick, D. K., Pfleger, F. L., Grau, C. R. (2001). Aphanomyces root rot. In: Kraft JM, Pfleger FL (eds) Compendium of pea diseases and pests, 2nd ed. APS Press, St. Paul, MN. p 913.

Pilet-Nayel, M. L., Coyne, C., Hamon, C., Lesne´, A., Le Goff, I., Esnault, R., Lecointe, R., Roux-Duparque, M., McGee, R., Mangin, P., McPhee, K., Moussart, A., and Baranger, A. (2007). Understanding genetics of partial resistance to Aphanomyces root rot in pea for new breeding prospects. In: Third international Aphanomyces workshop on legumes, 07–09 November 2007, Rennes, France, p 36.

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. Phytopathol. 95: 1287-1293.

Postnikova, O. A., Shao, J., and Nemchinov, L. G. (2013). Analysis of the alfalfa root transcriptome in response to salinity stress. Plant Cell Physiol. 54:1041-55. doi: 10.1093/pcp/pct056.

Punja, Z. K., Wan, A., Goswami, R. S., Verma, N., Rahman, M., Barasubiye, T., Seifert, K. A., and Lévesque, C. A. (2007). Diversity of Fusarium species associated with discolored ginseng roots in British Columbia. Can. J. Plant Pathol. 29:4, 340-353, doi: 10.1080/07060660709507480.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https: //www.R-project.org/.

Rasiukevičiūtė, N., Supronienė, S., Kelpsiene, J., Svegzda, P., Kadžienė, G., Šneideris, D., Ivanauskas, A., and Treikale, O. (2019). Susceptibility of non-cereal crops to Fusarium graminearum complex and their role within cereal crop rotation as a source of inoculum for Fusarium head blight. Span. J. Agric. Res., 16: 1012.

Ray, S., and Satya, P. (2014). Next generation sequencing technologies for next generation plant

breeding. Front. Plant Sci. 5: 367. https://doi.org/10.3389/fpls.2014.00367

Robert-Seilaniantz A, Navarro L, Bari R, Jones JD (2007) Pathological hormone imbalances. Curr Opin Plant Biol 10: 372–379

Roux-Duparque, M., Boitel, C., Decaux, B., Moussart, A., Alamie, J., Pilet- Nayel, M. L., and Muel, F. (2004). Breeding peas for resistance to Aphanomyces root rot: current main outputs of three breeding programmes. In: Proceedings of the 5th European conference on grain legumes, Dijon, France, p 133.

Ruan, J., Zhou, Y., Zhou, M., Yan, J., Khurshid, M., Weng, W., Cheng, J., & Zhang, K. (2019). Jasmonic Acid Signaling Pathway in Plants. International journal of molecular sciences, 20(10), 2479. https://doi.org/10.3390/ijms20102479

Safarieskandari, S., Chatterton, S., and Hall, L. (2020). Pathogenicity and host range of Fusarium species associated with pea root rot in Alberta, Canada. Can. J. Plant Pathol. 43: 162-171. doi: 10.1080/07060661.2020.1730442.

Sahu, P. K., Sao, R., Mondal, S., Vishwakarma, G., Gupta, S. K., Kumar, V., Singh, S., Sharma, D., and Das, B. K. (2020). Next Generation Sequencing Based Forward Genetic Approaches for Identification and Mapping of Causal Mutations in Crop Plants: A Comprehensive Review. Plants. 9:1355. https://doi.org/10.3390/plants9101355

Sarwar, M., Kirkegaard, J., Wong, P., and Desmarchelier, J. (1998). Biofumigation potential of brassicas. Plant and Soil. 201: 103-112.

Sato, M., Tsuda, K., Wang, L., Coller, J., Watanabe, Y., Glazebrook, J., & Katagiri, F. (2010). Network Modeling Reveals Prevalent Negative Regulatory Relationships between Signaling Sectors in Arabidopsis Immune Signaling. PLoS Pathog. 6:e1001011

Sato, S., Nakamura, Y., Kaneko, T., Asamizu, E., Kato, T., Nakao, M. Sasamoto, S., Watanabe, A., Ono, A., Kawashima, K., Fujishiro, T., Katoh, M., Kohara, M., Kishida, Y., Minami, C., Nakayama, S., Nakazaki, N., Shimizu, Y., Shinpo, S., Takahashi, C., Wada, T., Yamada, M., Ohmido, N., Hayashi, M., Fukui, K., Baba, T., Nakamichi, T., Mori, H., and Tabata, S. (2008). Genome structure of the legume, Lotus japonicus. DNA Res. 15: 227–239.

Sauvage, H., Moussart, A., Bois, F., Tivoli, B., Barray, S., and Laval, K. (2007). Development of a molecular method to detect and quantify Aphanomyces euteiches in soil. FEMS Microbiol Lett. 273: 64-9. doi: 10.1111/j.1574-6968.2007.00784.x.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., and Nelson, A. (2019). The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 3: 430–439. doi: 10.1038/s41559-018-0793-y.

Schlegel, A. J., and Havlin, J. L. (1997). Green Fallow for the Central Great Plains. Agron. J. 89: 762-767. https://doi.org/10.2134/agronj1997.00021962008900050009x.

Schmutz, Jeremy, Cannon, Steven B., Schlueter, Jessica, Ma, Jianxin, Mitros, Therese, Nelson, William, Hyten, David L., Song, Qijian, Thelen, Jay J., Cheng, Jianlin, Xu, Dong, Hellsten, Uffe, May, Gregory D., Yu, Yeisoo, Sakura, Tetsuya, Umezawa, Taishi, Bhattacharyya, Madan K., Sandhu, Devinder, Valliyodan, Babu, Lindquist, Erika, Peto, Myron, Grant, David, Shu, Shengqiang, Goodstein, David, Barry, Kerrie, Futrell-Griggs, Montona, Abernathy, Brian, Du, Jianchang, Tian, Zhixi, Zhu, Liucun, Gill, Navdeep, Joshi, Trupti, Libault, Marc, Sethuraman, Anand, Zhang, Xue-Cheng, Shinozaki, Kazuo, Nguyen, Henry T., Wing, Rod A., Cregan, Perry, Specht, James, Grimwood, Jane, Rokhsar, Dan, Stacey, Gary, Shoemaker, Randy C., and Jackson, Scott A. (2012). Genome sequence of the palaeopolyploid soybean. Nature. 463: 178–183.

Schnable, P. S., Ware, D., Fulton, R. S., Stein, J. C., Wei, F., Pasternak, S., Liang, C., Zhang, J., Fulton, L., Graves, T. A., Minx, P., Reily, A. D., Courtney, L., Kruchowski, S. S. et al. (2018). The B73 maize genome: complexity, diversity, and dynamics. Science. 326: 1112-1115. doi: 10.1126/science.1178534.

Schneider, K. A., Grafton, K. F., and Kelly, J. D. (2001). QTL analysis of resistance to Fusarium root rot in Bean. Crop Sci. 41: 535-542.

Schwartz, H. F., Steadman, J. R., Hall, R., and Forster, R. L. (2005). Compendium of bean diseases. 2nd ed. APS Press, St. Paul, MN. 109 pp.

Sekhon, R.S., Briskine, R.V., Hirsch, C.N., Myers, C.L., Springer, N.M., Buell, C.R., de Leon, N., & Kaeppler, S.M. (2014). Correction: Maize Gene Atlas Developed by RNA Sequencing and

Comparative Evaluation of Transcriptomes Based on RNA Sequencing and Microarrays. PLoS ONE, 9: 10. doi:10.1371/annotation/0444d495-9231-4097-abe0-4750b9045971.

Sekizaki, H., Yokosawa, R., Chinen, C., Adachi, H., and Yamane, Y. (1993). Studies on zoospore attracting activity: Synthesis of isoflavones and their attracting activity to Aphanomyces euteiches zoospore. Biol. Pharm. Bull. 16: 698-701.

Serafin-Andrzejewska, M., Kozak, M., and Kotecki, A. (2021). Effect of Pod Sealant Application on the Quantitative and Qualitative Traits of Field Pea (*Pisum sativum* L.) Seed Yield. Agriculture. 11: 645. https://doi.org/10.3390/agriculture11070645.

Severin, A.J., Woody, J.L., Bolon, Y., Joseph, B., Diers, B.W., Farmer, A.D., Muehlbauer, G.J., Nelson, R.T., Grant, D.M., Specht, J.E., Graham, M.A., Cannon, S.B., May, G.D., Vance, C.P., and Shoemaker, R.C. (2010). RNA-Seq Atlas of Glycine max: A guide to the soybean transcriptome. BMC Plant Biol. 10: 160 - 160.

Shaffer, C. (2007). Next-generation sequencing outpaces expectations. Nat. Biotechnol. 25: 149. doi: 10.1038/nbt0207-149.

Shehata, M. A., Davis, D. W., and Pfleger, F. L. (1983). Breeding for resistance to *Aphanomyces euteiches* root rot and *Rhizoctonia solani* stem rot in peas. J. Am. Hortic. Sci. 108: 1080-1085.

Shendure, J., and Ji, H. (2008). Next-generation DNA sequencing. Nat Biotechnol 26: 1135–1145.

Shivas, R. G. (1989). Fungal and bacterial disease of plants in Western Australia. J. R. Soc. West. Aust. 72: 1-62.

Sindhu, A., Ramsay, L., Sanderson, L. A., Stonehouse, R., Li, R., Condie, J., Shunmugam, A. S. K., Liu, Y., Jha, A. B., Diapari, M., Burstin, J., Aubert, G., Tar'an, B., Bett, K. E., Warkentin, T. D., and Sharpe, A. G. (2014). Gene-based SNP discovery and genetic mapping in pea. Theor. Appl. Genet. 127: 2225-2241.

Slinkard, A. E., van Kessel, C., Feindei, D. E., Aii-Khan, S. T., and Park, R. (1994). Addressing farmers' constraints through on-farm research: peas in western Canada. In: F. J. Muehibauer and W. J. Kaiser (eds.) Expanding the production and use of cool season food legumes. Kluwer Academic Publisher. The Netherlands. pp. 877-889.

Smith, P. G., and Walker, J. C. (1941). Certain environmental and nutritional factor affecting Aphanomyces root rot of garden pea. J. Agric. Res. 63: 1-20.

Smýkal, P., Aubert, G., Burstin, J., Coyne, C.J., Ellis, N., Flavell, A.J., Ford, R., Hýbl, M., Macas, J., Neumann, P., McPhee, K., Redden, R.R., Rubiales, D., Weller, J.L., and Warkentin, T. (2012). Pea (*Pisum sativum* L.) in the Genomic Era. Agronomy. 2:74–115.doi: 10.3390/agronomy2020074

Solaiman, Z., Colmer, T. D., Loss, S. P., Thomson, B. D., and Siddique, K. H. M. (2007). Growth responses of cool-season grain legumes to transient waterlogging. Aust. J. Agric. Res. 58: 406–412. doi: 10.1071/AR06330.

Son, H. Kim, M. G., Min, M., Seo, Y. S., Lim, J. Y. Choi, G. J. Kim, J. C., Chae, S. K., and Lee, Y. W. AbaA regulates conidiogenesis in the ascomycete fungus Fusarium graminearum. PLoS One 8: e72915. doi: 10.1371/journal.pone.0072915 .

Su, Y., Gabrielle, B., Beillouin, D., and Makowski, D. (2021). High probability of yield gain through conservation agriculture in dry regions for major staple crops. Sci. Rep. 11: 3344. doi:10.1038/s41598-021-82375-1

Sudheesh, S., Sawbridge, T.I., Cogan, N.O., Kennedy, P., Forster, J.W., and Kaur, S. (2015). De novo assembly and characterisation of the field pea transcriptome using RNA-Seq. BMC Genomics 16: 611. https://doi.org/10.1186/s12864-015-1815-7

Sundheim, L. (1972). Physiologic specialization in Aphanomyces euteiches. Physiol. Plant Pathol. 2: 301–306. doi: 10.1016/0048-4059(72)90013-6.

Tang, R. J., Luan, M., Wang, C., Lhamo, D., Yang, Y., Zhao, F. G., Lan, W. Z., Fu, A. G., and Luan, S. (2019). Plant Membrane Transport Research in the Post-genomic Era. Plant Commun. 1: 100013. https://doi.org/10.1016/j.xplc.2019.100013

Tar'an, B., Warkentin, T., Somers, D. J., Miranda, D., Vandenberg, A., Blade, S., Woods, S., Bing, D., Xue, A., DeKoeyer, D., Penner, G. (2003). Quantitative trait loci for lodging resistance, plant height and partial resistance to mycosphaerella blight in field pea (*Pisum sativum* L.). Theor. Appl. Genet. 107: 1482-91. doi: 10.1007/s00122-003-1379-9. PMID: 12920512.

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., Warkentin, T. D., Brune, D., Marget, P., Le Paslier, M., Aubert, G., and Burstin, J. (2015). Development of two major resources for pea genomics: the GenoPea 13.2K SNP Array and a high‐density, high‐resolution consensus genetic map. The Plant Journal 84: 1257-1273.

Thompson, R. S., Aveling, T. A. S., and Prieto, B. R. (2012). A new semi-selective medium for *Fusarium graminearum*, *F. proliferatum*, *F. subglutinans* and *F. verticillioides* in maize seed. South African Journal of Botany. 84. 10.1016/j.sajb.2012.10.003.

Tonnberg, V., Liljeroth, E., and Stegmark, R. (2016). Evaluation of resistance against Fusarium root rot in peas. Swedish Univ. of Ag. Sci.

Trapnell, C., Roberts, A., Goff, L.A., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., & Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. 7: 562–578.

Trinidad, T. P., Mallillin, A. C., Loyola, A. S., Sagum, R. S., and Encabo, R. R. (2010). The potential health benefits of legumes as a good source of dietary fibre. Br. J. Nutr. 103: 569-74.

Tu, J. C., and Findlay, W. 1. (1986). The effects of different green manure crops and tillage practices on pea root rots. Proc. Br. Crop Prot. Conf. Pest Dis. In press.

Tyler, B. M. (2007). *Phytophthora sojae:* root rot pathogen of soybean and model oomycete. Mol. Plant Pathol. 8: 1-8. doi: 10.1111/j.1364-3703.2006.00373.x. PMID: 20507474.

Van der Lee, T., Zhang, H., van Diepeningen, A., and Waalwijk, C. (2015). Biogeography of *Fusarium graminearum* species complex and chemotypes: A review. Food Addit. Contam. 32: 453–463.

Vandemark, G. J., Barker, B. M., and Gritsenko, M. A. (2002). Quantifying Aphanomyces euteiches in alfalfa with a luorescent polymerase chain reaction assay. Phytopathology .92: 265–272.

VanEtten, H. D. (1978). Identification of additional habitats of Nectriahaematococca mating population VI. Phytopathology. 68: 1552–1556.

Varshney, R.K., Chen, W., Li, Y., Bharti, A.K., Saxena, R.K., Schlueter, J.A., Donoghue, M.T., Azam, S., Fan, G., Whaley, A.M., Farmer, A.D., Sheridan, J.L., Iwata, A., Tuteja, R., Penmetsa, R.V., Wu,

W., Upadhyaya, H.D., Yang, S., Shah, T.M., Saxena, K., Michael, T.M., McCombie, W.R., Yang, B., Zhang, G., Yang, H., Wang, J., Spillane, C., Cook, D.R., May, G.D., Xu, X., and Jackson, S.A. (2012). Draft genome sequence of pigeonpea (*Cajanus cajan*), an orphan legume crop of resource-poor farmers. Nat. Biotech. 30:83–89.

Varshney, R.K., Song, C., Saxena, R.K., Azam, S., Yu, S., Sharpe, A.G., Cannon, S.B., Baek, J., Rosen, B.D., Tar'an, B., Millán, T., Zhang, X., Ramsay, L., Iwata, A., Wang, Y., Nelson, W., Farmer, A.D., Gaur, P.M., Soderlund, C., Penmetsa, R.V., Xu, C., Bharti, A.K., He, W., Winter, P., Zhao, S., Hane, J.K., Carrasquilla-Garcia, N., Condie, J.A., Upadhyaya, H.D., Luo, M., Thudi, M., Gowda, C.L., Singh, N.P., Lichtenzveig, J., Gali, K.K., Rubio, J., Nadarajan, N., Doležel, J., Bansal, K.C., Xu, X., Edwards, D., Zhang, G., Kahl, G., Gil, J., Singh, K.B., Datta, S.K., Jackson, S.A., Wang, J., & Cook, D.R. (2013). Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. Nat. Biotech. 31: 240–246.

Voorrips, R. E. (2002). MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs. J. Hered. 93: 77–78. https: //doi.org/10.1093/jhered/93.1.77

Wakelin, S., Walter, M., Jaspers, M.V., and Stewart, A. (2011). Biological control of Aphanomyces euteiches root-rot of pea with spore-forming bacteria. Australasian Plant Pathology. 31: 401–407https://doi.org/10.1071/AP02051.

Wang, N. (2020). Quality of western Canadian peas – 2020. Canadian Grain Commission. Available at: https://www.grainscanada.gc.ca/en/grain-research/export-quality/pulses/peas/2020/

Wang, S., Basten, C. J., and Zeng, Z. B. (2012). Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Wang, Z., Gerstein, M., and Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. 10:57–63.

Wang. Y., VandenLangenberg, K., Wen, C, Wehner, T. C., and Weng, Y. (2018). QTL mapping of downy and powdery mildew resistances in PI 197088 cucumber with genotyping-by-sequencing in RIL population. Theor. Appl. Genet. 131: 597-611. doi: 10.1007/s00122-017-3022-1. Epub 2017

Nov 20. PMID: 29159421.

Weeden, N. F., McGee, R., Grau, C. R., and Muehlbauer, F. J. (2000). A gene influencing tolerance to common root rot is located on linkage group IV. Pisum Genet. 32:53–55

Weeden, N. F., McGee, R., Grau, C. R., and Muehlbauer, F. J. (2000). A gene influencing tolerance to common root rot is located on linkage group IV. Pisum Genet. 32: 53-55

White, T. J., Bruns, T., Lee, S., and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNS genes for phylogenetics. Pages 315-322 in: PCR Protocols: A guide to methods and applications. M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White, eds. Academic Press, San Diego, CA.

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. doi: 10.1023/A:1013171217610.

Wicker, E., Hullé, M., and Rouxel, F. (2001). Pathogenic characteristics of isolates of *Aphanomyces euteiches* from pea in France. Plant Pathol. 50: 433-442.

Wickham, H. (2011). The Split-Apply-Combine Strategy for Data Analysis. J. Stat. Softw. 40: 1-29. URL http://www.jstatsoft.org/v40/i01/.

Wille, L., Messmer, M. M., Bodenhausen, N., Studer, B., and Hohmann, P. (2020). Heritable Variation in Pea for Resistance Against a Root Rot Complex and Its Characterization by Amplicon Sequencing. Front. Plant Sci. 11. doi: 10.3389/fpls.2020.542153

Williamson-Benavides, B. A, Sharpe, R. M., Nelson, G., Bodah, E. T., Porter, L. D., and Dhingra, A. (2021b). Identification of Root Rot Resistance QTLs in Pea Using *Fusarium solani* f. sp. pisi-Responsive Differentially Expressed Genes. Front Genet. 12:629267. doi: 10.3389/fgene.2021.629267.

Williamson-Benavides, B. A., and Dhingra, A. (2021a). Understanding Root Rot Disease in Agricultural Crops. Horticulturae. 7: 33.

Willsey, T. L., Chatterton, S., Heynen, M., and Erickson, A. (2018). Detection of interactions between the pea root rot pathogens Aphanomyces euteiches and Fusarium spp. using a multiplex qPCR

assay. 67:1912-1923. http://dx.doi.org/10.1111/ppa.12895

Wu, K. K., Xie, X. T., and Li, X. J. (1992). Study on the pathogen of root rot of pea in the central region of Gansu province. Gansu Nongye Daxue Xuebao. 27: 225-231.

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 doi: 10.1007/s00122-021-03871-6.

Wu, L. F., Chang, K. F., Conner, R. L., Strelkov, S. E., Fredua-Agyeman, R., Hwang, S. F., and Feindel, D. (2018). *Aphanomyces euteiches*: A Threat to Canadian Field Pea Production. Engineering 4: 542-551

Wu, L. F., Chang, K. F., Fu, H., Aker, I., Li, N., Hwang, S. F., Turnbull, G. D., Strelkov, S. E., and Feindel, D. (2017). The occurrence of and microorganisms associated with root rot of field pea in Alberta in 2016. Can. Plant Dis. Surv. 97: 193-195

Wu, L. F., Chang, K. F., Hwang, S. F., Conner, R. L., Fredua-Agyeman, R., Feindel, D., and Strelkov, S. E. (2019). Evaluation of host resistance and fungicide application as tools for the management of root rot of field pea caused by *Aphanomyces euteiches*. Crop J. 7: 38-48

Wu, P., Xie, J., Hu, J., Qiu, D., Liu, Z., Li, J., Li, M., Zhang, H., Yang, L., Liu, H., Zhou, Y., Zhang, Z., and Li, H. (2018). Development of Molecular Markers Linked to Powdery Mildew Resistance Gene Pm4b by Combining SNP Discovery from Transcriptome Sequencing Data with Bulked Segregant Analysis (BSR-Seq) in Wheat. Front Plant Sci. 9:95. doi: 10.3389/fpls.2018.00095.

Wu, Y., Bhat, P. R., Close, T. J., and Lonardi, S. (2008). Efficient and accurate construction of genetic linkage maps from the minimum spanning tree of a graph. PLoS genetics 4: e1000212. https: //doi.org/10.1371/journal.pgen.1000212

Xu, Y., and Crouch, J. H (2008). Marker-Assisted Selection in Plant Breeding: From Publications to Practice. Crop Sci. 48: 391-407. DOI: 10.2135/cropsci2007.04.0191

Xue, A .G., Tuey, H. J., and Mathur, S. (1998). Diseases of field pea in Manitoba in 1997. Can. Plant Dis. Surv. 78: 97–98

Xue, A. G. (2003). Biological Control of Pathogens Causing Root Rot Complex in Field Pea Using Clonostachysrosea Strain ACM941. Phytopathology. 93: 329-35. doi: 10.1094/PHYTO.2003.93.3.329.

Xue, A. G., Chen, Y., Seifert, K., Guo, W., Blackwell, B. A., Harris, L. J., and, Overy, D. P. (2019). Prevalence of Fusarium species causing head blight of spring wheat, barley and oat in Ontario during 2001–2017. Can. J. Plant Pathol. 41: 392-402. doi: 10.1080/07060661.2019.1582560

Xue, A.G., Tuey, H.J., and Platford, R.G. (2000). Diseases of field pea in Manitoba in 1999. Can. Plant Dis. Surv. 80: 110–111.

Yang, S.S., Tu, Z.J., Cheung, F., Xu, W.W., Lamb, J.F., Jung, H., Vance, C.P., and Gronwald, J.W. (2011). Using RNA-Seq for gene identification, polymorphism detection and transcript profiling in two alfalfa genotypes with divergent cell wall composition in stems. BMC Genomics 12 : 199. https://doi.org/10.1186/1471-2164-12-199

Yang, Y., Zhang, X., Wu, L., Zhang, L., Liu, G., Xia, C., Liu, X., & Kong, X. (2021). Transcriptome profiling of developing leaf and shoot apices to reveal the molecular mechanism and co-expression genes responsible for the wheat heading date. BMC Genomics 22: 468. https://doi.org/10.1186/s12864-021-07797-7

Yin, L., Zhang, H., Tang, Z., Xu, J., Yin, D., Zhang, Z., Yuan, X., Zhu, M., Zhao, S., Li, X., and Liu, X. (2021). rMVP: A Memory-efficient, Visualization-enhanced, and Parallel-accelerated tool for Genome-Wide Association Study. Genom. Proteom. Bioinform. 1: S1672-0229(21)00050-4. doi: 10.1016/j.gpb.2020.10.007.

Yokosawa, R., Kuninaga, S., and Teranaka, M. (1974). Note on pea root rot fungus, *Aphanomyces euteiches* Drechs in Japan. Ann. Phytopathol. Soc. Japan. 40: 454-457(in Japanese).

Yoshida, H., Tomiyama, Y., Saiki, M., and Mizushina, Y. (2007). Tocopherol Content and Fatty Acid Distribution of Peas (*Pisum sativum* L.). J. J. Am. Oil. Chem. Soc. 84**:** 1031-1038.

Yu, F., Zhang, X., Huang, Z., Chu, M., Song, T., Falk, K.C., Deora, A., Chen, Q., Zhang, Y., McGregor, L., Gossen, B.D., Mcdonald, M.R., and Peng, G. (2016). Identification of Genome-Wide Variants and Discovery of Variants Associated with Brassica rapa Clubroot Resistance Gene

Rcr1 through Bulked Segregant RNA Sequencing. PLOS ONE 11: e0153218. https://doi.org/10.1371/journal.pone.0153218

Yu, F., Zhang, X., Peng, G., Falk, K. C., Strelkov, S. E., and Gossen, B. D. (2017). Genotyping-by-sequencing reveals three QTL for clubroot resistance to six pathotypes of Plasmodiophorabrassicae in Brassica rapa. Scientific reports. 7:4516. https://doi.org/10.1038/s41598-017-04903-2.

Zaidi, PH., Rashid, Z., Vinayan, M. T., Almeida, G. D., Phagna, R. K., and Babu, R. (2015). QTL Mapping of Agronomic Waterlogging Tolerance Using Recombinant Inbred Lines Derived from Tropical Maize (*Zea mays* L) Germplasm. PLoS ONE 10: e0124350. doi: 10.1371/journal.pone.0124350

Zaman, M. S. U., Malik, A. I., Kaur, P., Ribalta, F. M., and Erskine, W. (2019). Waterlogging Tolerance at Germination in Field Pea: Variability, Genetic Control, and Indirect Selection. Front. Plant Sci. 10: 953. doi: 10.3389/fpls.2019.00953.

Zhang, D., Hendricks, D. G., Mahoney, A. W., Cornforth, D. P. (1985). Bioavailability of Iron in Green Peas, Spinach, Bran Cereal and Cornmeal Fed to Anemic Rats. J. Food Sci. 50: 426-428.

Zhao, S., Fung-Leung, W-P., Bittner, A., Ngo, K., Liu, X. (2014). Comparison of RNA-Seq and microarray in transcriptome profiling of activated T cells. PLOS ONE. 9:1–13. doi:10.1371/journal.pone.0078644

Zhao, Y., Xia, Q., Yin, Y., and Wang, Z. (2016). Comparison of Droplet Digital PCR and Quantitative PCR Assays for Quantitative Detection of Xanthomonas citri Subsp. citri. PLoS One. 11: e0159004.

Zhou, Q., Chen, Y., Yang, Y., Ahmed, H., Hwang, S. F., and Strelkov, S. E. (2014). Effect of inoculum density and quantitative PCR-based detection of *Rhizoctonia solani* AG-2-1 and Fusarium avenaceum on canola. Crop Prot. 59: 71-77.

Zhu, Q., Stephen, S., Kazan, K., Jin, G., Fan, L., Taylor, J.M., Dennis, E.S., Helliwell, C.A., and Wang, M. (2013). Characterization of the defense transcriptome responsive to Fusarium oxysporum-infection in Arabidopsis using RNA-seq. Gene. 512: 259-66.

Ziesman, B., Bawolin, C., Sliva, T., Hartley, S., Hladun, M., Fernandez, M. R., and Peru, C. (2019). Fusarium head blight in barley in Saskatchewanin 2018. Can. Plant Dis. Surv. 99: 69-70.

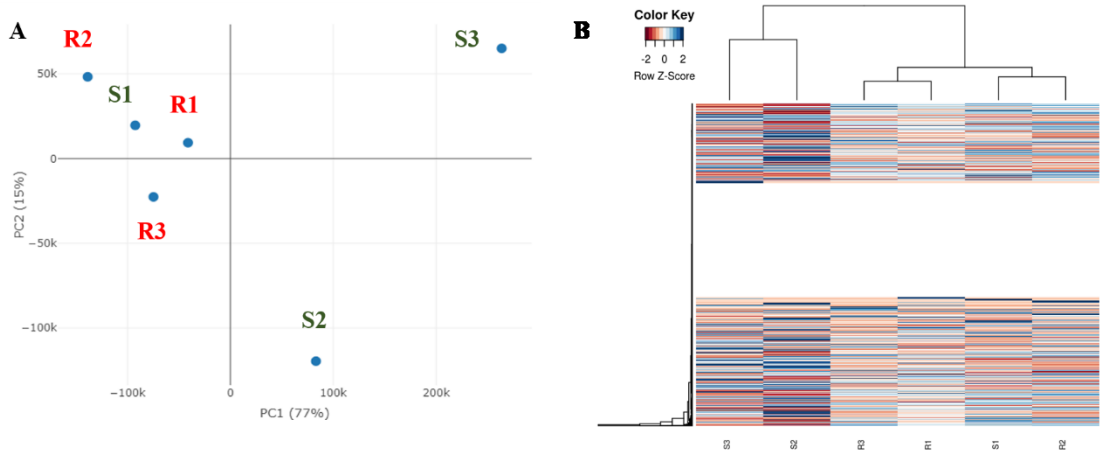
Zipfel, C. (2009). Early molecular events in PAMP-triggered immunity. Curr Opin Plant Biol. 12: 414–20.

Zitnick-Anderson, K. K., Pasche, J. S., Gargouri-Jbir, T., and Kalil, A. K. (2020). First report of *Aphanomyces euteiches* causing Aphanomyces root rot on lentil in North Dakota. Plant Dis. 104, 1876.

Zitnick-Anderson, K., Simons, K., and Pasche, J. S. (2018). Detection and qPCR quantification of seven Fusarium species associated with the root rot complex in field pea. Can. J. Plant Pathol. 40: 261-271. doi: 10.1080/07060661.2018.1429494

Zohary, D., and Hopf, M. (1973). Domestication of pulses in the Old World. Science 182: 887-894.

Appendix



Supplementary Figure 1. (A) Principal component analysis (PCA) of six pea RNA samples representing a total of 44,757 genes. The first dimension explained 77% of the total variance (x-axis) and the second dimension accounted for 15% of the total variance (y-axis). The red-colored letters indicate three samples from Aphanomyces root rot-resistant (R) bulks, while the green letters indicate the susceptible (S) bulks; (B) Hierarchical clustering of the 44,757 pea genes for three replicates of the R and S bulks, with blue and red representing up-regulated and down-regulated genes, respectively