GENETIC ANALYSIS AND MOLECULAR MAPPING OF GENES RESISTANT TO DIDYMELLA PISI IN PEAS

by

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DEDICATION

I dedicate this thesis to my father, Yagya and my mother, Bhawana, without whom I would not be here. Thank you for everything you have done for me.

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ABSTRACT

Ascochyta Blight, a seed-borne and foliar disease of peas poses a significant threat to its production. The disease is caused by four necrotrophic fungal pathogens known as the Ascochyta complex, including *Didymella pisi, D. pinodes, D. pinodella*, and *Phoma koolunga*, and *D. pisi* is the most prevalent species in Montana, causes up to 75% yield loss. Current disease management strategies include crop rotation, fungicides, and limited use of resistant cultivars due to the lack of available resistance resources for breeding. From a high-throughput screening, we identified the cultivar "Fallon" was immune to *D. pisi*. Two mapping populations derived from crosses between Fallon and the susceptible cultivar, "Stirling", have been developed. Through progeny testing and genetic analysis, two recessive genes in Fallon are required to confer resistance against *D. pisi* isolate '22-P2033', and only one recessive gene is required for resistance against *D. pisi* isolate '10728'. The resistance genes required for *D. pisi* isolate '22-P2033' were mapped on chromosomes I and V, and the resistance gene required for resistance against *D. pisi* isolate '10728' was mapped on chromosome V. Additional polymorphisms have been identified for future marker development to assist the selection of the resistance gene. The research facilitates resistance breeding to Ascochyta Blight and paves the path for future marker development.

INTRODUCTION AND LITERATURE REVIEW

Introduction

Ascochyta blight is one of the most economically important diseases of peas. It is caused by a complex of necrotrophic fungal species, i.e., *Didymella pisi*, *D. pinodes, D. pinodella*, and *Phoma koolunga*, which might occur individually or together in peas (Skoglund et al., 2011). The damage is more devastating when the species are present together. Resistance to one species does not guarantee resistance to other species of fungi that cause Ascochyta. The dominant species varies across regions worldwide. The northern states of America, Montana, North Dakota, and Washington are the highest pea-growing states (USDA, 2023). Ascochyta blight affects up to 70% of pea production in these states (McDonald & Peck, 2009). *Didymella pisi* is the most prevalent species in Montana (Annan et al., 2023). The current management practices include the use of synthetic fungicides, crop rotation, and very few moderately resistant varieties like Century, Voyageur and Carneval (Kumar et al., 2017; Wang et al., 2000). From a high-throughput screening of resistance against Ascochyta blight, Fallon is only variety identified as completely immune to *D. pisi*, but there has not been any research on its mechanism of resistance (Annan et al., 2023). Thus, it is very important to study the genetic mechanism of resistance to Ascochyta blight in peas. This thesis focuses on genetic analysis and mapping of the gene conferring resistance to *D. pisi* in peas.

Objectives:

1. Genetic analysis of resistance to *D. pisi* in Fallon.
2. Molecular mapping of the resistant genes to pea chromosomes.

Literature Review

Introduction to peas

Peas are cool-season legumes that belong to the agriculturally important family Leguminosae (Fabaceae), which is known for its diversity, with almost 770 genera and more than 19,500 species in the family (Graham & Vance, 2003). There are six species of peas: *Pisum sativum* (field pea), *P. elatius* (Mediterranean pea), *P. arvense* (field pea), *P. abyssinicum* (Abyssinian pea), *P. humile* (dwarf pea), and *P. fulvum* (red, yellow pea) (Ram et al., 2021). The most commonly grown and consumed pea in the world is the field pea (or dry pea), which is a diploid plant with a chromosome number 2n=14. Field pea is an annual herbaceous plant that grows up to 3 meters long. The plants have alternate, pinnately compound leaves with large leaf-like stipules, with racemose inflorescences that emerge from the axils of their leaves, giving white to reddish-purple flowers. They have single to multiple podded pods that contain five to ten seeds each and grow up to 10 cm long (Yang et al., 2022).

Economic importance

Pea is one of the earliest domesticated crops and it has been an important part of agriculture from early farming systems to modern pulse production (Zohary et al., 1973). Pea is economically important due to its highly versatile nature and a wide range of applications, both in the form of fresh or dried seeds. The fresh and dried peas are used as a vegetable, in soup, stew or salads. Besides a food crop it has also been used as a forage crop, green manure, cover crop, hay and silage (Holdsworth et al., 2017). In many parts of the world, pea remains as the most affordable source of plant-based protein, with protein content ranging from 12.6% to 27.6%

on a dry-weight basis (Thavarajah et al., 2023). Pea protein has been proven to have a lot of health benefits. It is basically a source of all nutrients, including all essential amino acids, important vitamins, and minerals. Vitamins A, D, E, K, B2, and B6 can be found in peas, which support a healthy immune system, bone health, and neurological function. The important minerals such as Iron, Potassium, Magnesium, and Zinc, help in lowering blood pressure and maintaining good cardiovascular health (Wu et al., 2023). Besides as a harvested crop, pea is important in cropping system as it fixes atmospheric nitrogen into the soil in symbiosis with the rhizobia (Lupwayi & Kennedy, 2007). This reduces the requirement for synthetic nitrogen fertilizers in the field, and in rotational cropping, subsequent crops also benefit from the residual nitrogen effects. In the recent years, with the increasing focus on limiting the use of synthetic fertilizers, maintaining soil health, organic agriculture and sustainable plant protein production, pea has become an important crop in both farming systema and food industries (Karkanis et al., 2016).

Pea production and distribution

Archeological evidence suggests peas originated from the Mediterranean, primarily in the Middle East region. The domestication of peas started from Middle East, slowly moved to Europe and spread to Asia, North America, Australia, and other regions with suitable climates. Currently, pea is widely grown in Europe, North America, Canada, Australia, China, Russia and India. The global dry pea production was 14,157,952 tons in 2024 which was cultivated in nine-million-hectare area (FAO, 2026). China was the largest pea producer, with 1.49 million tons in 2024, followed by India and United States (FAO, 2026). The USA was the third highest dry pea producer

in the world with 756.550 tons of dry pea production in over 0.98 million acres (FAO, 2026). The pea production in USA raised to 821,002 tons in 2025, with the northern states, Montana, North Dakota, and Washington being the leaders of pea production in the USA. More than 50% of the total pea production in USA, was from Montana, i.e., 449,056 tons, followed by North Dakota and Washington at 272, 551 tons and 54, 431 tons respectively (World Population Review, 2026).

Pea Genome

The garden pea holds historical importance as the model organism in Gregor Mendel's experiments, done in the 19th century, which established the foundation of contemporary genetics. Despite this importance, pea genomics advancement has trailed behind those of other crops for many years due to the complexity of the pea genome. Peas are a diploid species (2n = 14) with seven chromosomes and an estimated size of 4.45 Gb, which makes pea genome one of the largest among the leguminous family (Ellis et al., 1998). As a reference, the pea genome is double the size of the human genome and nearly ten times larger than that of Arabidopsis thaliana. Additionally, almost 80% of the pea genome is composed of repetitive sequences, mostly retrotransposons, which has caused a major challenge for assembly and annotations (Smýkal et al., 2012).

A major breakthrough occurred when the first ever high-quality reference genome was published in 2019 by Kreplak and his colleagues. It was based on the French spring cultivar Cameor. They assembled the 88% of the pea genome into seven pseudochromosomes (Kreplak et al., 2019a). The researchers used whole-genome shotgun sequencing with long-read data, optical mapping, Hi-C scaffolding, and integration with genetic maps to assemble the pea genome. There are

roughly 44,791 protein-coding genes in the annotated reference genome, many of which are connected to disease resistance, symbiotic nitrogen fixation, and stress responses. This genetic resource accessibility has enabled the use of cutting-edge techniques, including molecular marker development, QTL mapping, candidate gene cloning, and genome-wide association studies (GWAS). Additionally, comparative genomics between peas and other legumes, such as Glycine max and Medicago truncatula, is now possible and has already shed light on genome synteny and evolution (Smýkal et al., 2015). Since 2019, the standard reference for peas has been the genome of Cameor (*Pisum sativum* v1a). This foundation is being expanded upon by current research, which aims to create pan-genomes and haplotype-resolved assemblies that integrate genetic diversity from wild and cultivated peas. The availability of this genomic data has revolutionized pea research by making it possible to use contemporary techniques, including candidate gene discovery, QTL mapping, genome-wide association studies, and marker development (Smýkal et al., 2015).

Challenges in pea production

Pea faces a wide range of biotic and abiotic threats. Among biotic threats, peas are prone to damage from pests and pathogens. Pea pathogens include various fungi, bacteria, nematodes, and viruses. Major fungal diseases include Powdery Mildew, Ascochyta blight, Rusts and wilts which lead up to 60% yield loss in plants (Kraft, 1994). Bacterial blight occurs sporadically but it has also led to severe yield losses (Taylor et al., 1996). Viral diseases such as Pea mosaic virus (PSbMV) and Bean yellow mosaic virus (BYMV) remain a significant constraint (Bos et al., 1988). Additionally, nematodes like the pea cyst nematode and root-knot nematodes also reduce

vigor and productivity (Briar et al., 2023). Only a few insects pose significant economic loss such as aphids, grasshoppers and lygus bugs. The extent of economic loss from these pathogens and pests varies across production regions.

Ascochyta Blight

Among the many pea diseases, Ascochyta blight is documented to cause the most devastating yield losses, with reports of up to 75% in peas (McDonald & Peck, 2009). This disease is also known as "black spot disease" and is distributed worldwide, in almost every pea-growing area (Liu et al., 2016). The pathogen was first described and named in 1830 by Libert from Europe (Skoglund et al., 2011a) and was first documented in 1911 in India's North-West Frontier Province, now part of Pakistan (Foresto et al., 2023). A complex of necrotrophic fungi, mainly *Didymella pisi, D. pinodes* (syn. *Mycosphaerella pinodes*), *D. pinodella,* and *Phoma koolunga*, are responsible for causing Ascochyta blight (Kropp et al., 2010). These pathogens may occur individually or in combination in any host. The species causing Ascochyta blight varies according to regions around the world (E. N. Annan et al., 2023). The disease symptoms and plant destruction are more severe when these species occur as together in plants (Davidson et al., 2009). The main symptom of Ascochyta Blight includes the development of necrotic lesions on the leaf surface, pods, tendrils, and stems of the plants. The lesion is brownish to purplish black in color surrounded by a tan border. The lesions are small and scattered in *D. pinodes* and bigger in size and patchy in *D. pisi*. The lesion contains spore-forming structures for the fungi (Skoglund et al., 2011a). As the disease progresses, early defoliation occurs. The disease also

hampers pod formation, leading to reduced seed production, with seeds that are discolored and distorted in shape (Foresto et al., 2023).

Pathogen biology and disease cycle

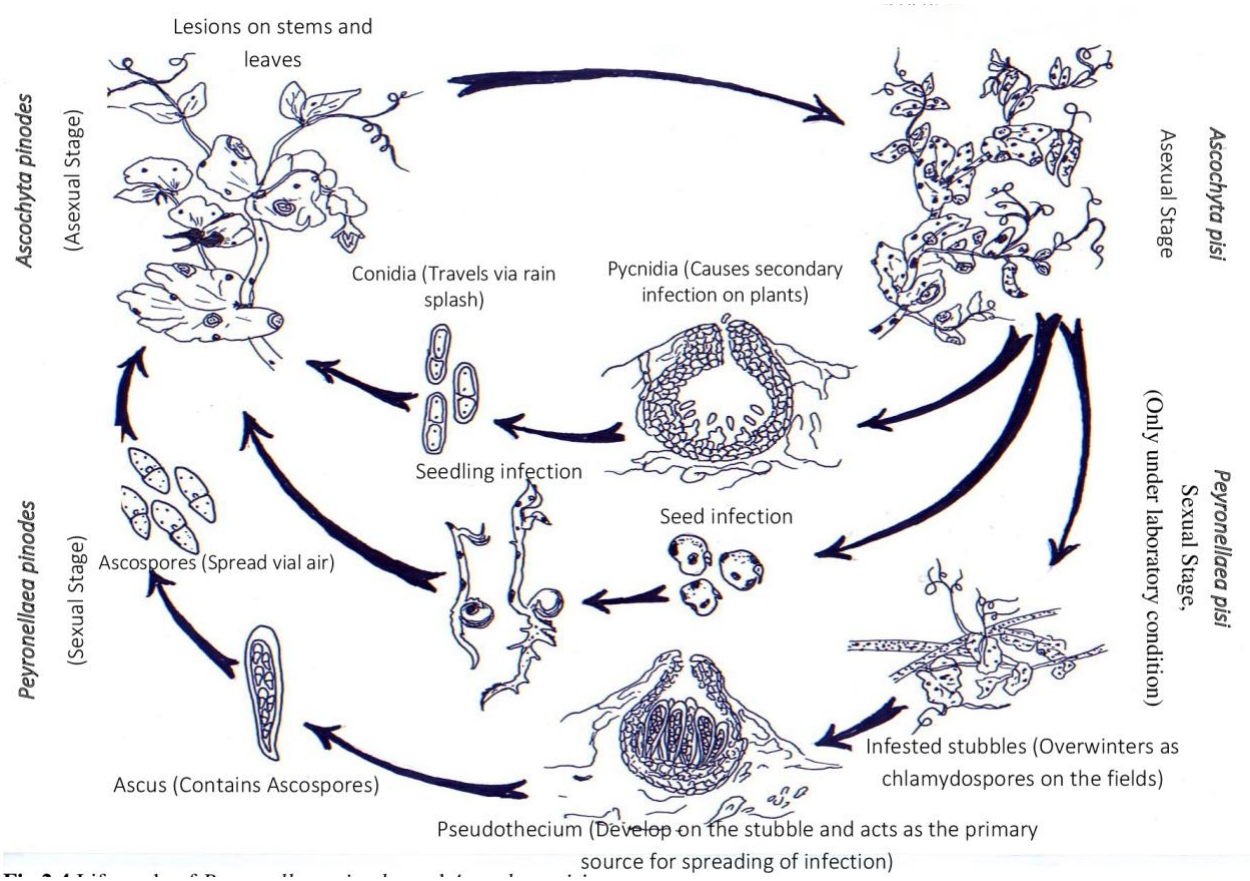


Figure 1: Life cycle of *D. pinodes* and *D. pisi* (Sivachandrakumar, 2016)

Ascochyta blight disease cycle starts with survival of pathogens between the cropping seasons, followed by the release of main inoculum, early infection of the new crop, and repeated cycles of secondary dissemination during favorable weather (Bretag et al., 2006). Infected

stubble, crop residue and infected seeds are the main ways of survival of the pathogen. The pathogen forms asexual fruiting body, pycnidia and sexual fruiting body, perithecia on infected tissue. The pycnospores produced by pycnidia and ascospores produced by perithecia are disseminated by air and water. Pycnospores usually travel a short distance by direct water movement whereas, ascospores are often released into the air and can spread farther than pycnospores (Davidson et al., 2013). Ascochyta blight infection starts when the fungal spores land on the plant's aerial surface like leaf or stem. Upon landing on the plant surface, the fungus secretes mucilaginous exudates to adhere the spore to the plant surface and keep it from drying out. In the presence of moisture, the spores form a germ tube within the first 12 hours. The germ tube penetration into the plant epidermis occurs through its tip, i.e., appressorium, within the first 24 hours (R. Singh et al., 2022). Then the fungal hyphae expand into the subepidermal space along with the pathogen secreting cell-wall-degrading enzymes like pectinase and xylanase. The symptoms of the disease start developing in 7 to 9 days and pycnidia is formed on the lesions. Under humid conditions, new pycnospores ooze out of pycnidia, and the secondary infection cycle continues (Pandey et al., 1987).

D. pinodes and *D. pinodella* produce darker-colored mycelium and yellowish spores, while *D. pisi* forms a lighter-colored mycelium and reddish to brownish spores. *D. pisi* spores are hyaline, straight or slightly curved, septate, somewhat constricted at the septum, rounded ends, and 10-16 ×3-4.5 µm. The spores of *D. pinodes* are hyaline, ellipsoid, guttulate, with one (very rarely more) septum, somewhat constricted at the septa, and measure 8–16 × 3-4.5 µm. The

hyaline, unicellular spores of *D. pinodella* measure 4.5-8 × 2-3 µm and are significantly smaller than those of *D. pisi* and *D. pinodes* (Skoglund et al., 2011).

Host-pathogen interaction

Ascochyta blight is a necrotic fungal disease in which the pathogen kills the plant tissue and feeds on decaying tissues to obtain nutrients. The pathogen secretes a cocktail of different compounds, including toxins, proteins, cell wall-degrading enzymes (CWDEs), or secondary metabolites to dissolve the structural barriers of the host by breaking down the plant cell walls and damaging the host cells. The Ascochyta blight causing species secretes the phytotoxin 'ascochitine' to create the characteristic brown or purplish-black necrotic lesions (Kan, 2006). Published evidence on *D. pinodes* shows a glycopeptide fungal elicitor from *D. pinodes* spore germination fluid triggering defense-related responses in the host (Kiba et al., 1999).

Upon detection of any microbe-associated molecular patterns (MAMPs) by surface localized receptors in the host, the host triggers an oxidative burst of Reactive Oxygen Species (ROS) to trigger the defense mechanism and strengthen cell wall by deposition of proteins and lignin in the cell wall. When specific fungi effectors are detected by NLR receptors, it triggers hypersensitive response (HR) and programmed cell death in host. But in case of necrotrophs, as they feed on dead tissues, necrotrophs benefit from programmed cell death to grow in host cell. (X. Wang et al., 2014). The primary pathway for plant defense is the Jasmonic Acid (JA) and Ethylene (ET), which activates defense genes and produce antimicrobial compounds like phytoalexins like pisatin for peas (Shao et al., 2021). There is no published evidence on receptors specifically related to Ascochyta blight yet. In order to physically prevent the fungus from

spreading to the stem, resistant cultivars strengthen their cell walls by increasing lignin content. Active defense against necrotic pathogens like Ascochyta blight causing species is most commonly associated with dominant resistance from the host, where dominant resistance genes enable the host to recognize the pathogen and activate defense responses. In contrast, passive resistance is often linked with recessive resistance, which results from the loss or alteration of host genes needed by the pathogen for infection. In such cases, the pathogen is unable to utilize essential host components required for penetration, colonization, or suppression of plant defenses.

Management of Ascochyta blight

The current management practices for Ascochyta blight are based on an integrated approach as no single approach is enough for disease control under field conditions. Effective management begins with acquiring disease-free seeds and using seed treatment to prevent primary inoculum from seeds. Under the cultural management practices, crop rotation is an important component of disease management as it reduces the survival of the pathogens on crop debris and soil, particularly in frequent pea growing areas (McDonald & Peck, 2009). Delayed sowing in June-July as compared to May has also shown to reduce disease severity by helping the crops avoid the period of high primary inoculum pressure. Chemical fungicides like Benomyl (Demirci et al., 2003) Thiram (Kachyatran et al., 1996) and Ethyl mercury (Lukashevich et al., 1958) are found to be effective against Ascochyta blight. Growers widely use chemical fungicides due to their easy access and availability. On the downside, the adverse use of chemical pesticides has aided in pathogen resistance, loss of useful micro-organisms from soil,

loss of beneficial bacteria and fungi. With the misuse of chemical pesticides on food crops, there has been proved to be deterioration in human health after consumption (Singh, 2018).

Currently, environmentally friendly management practices are being explored and promoted. According to research conducted in 2005 by Rajakumar et al., the biocontrol agent, *Chaetomium globosum*, was found to have a significant effect in controlling Ascochyta Blight when used as both pre- and post-inoculant spray, with 73.12% reduction in disease index. In another research, *Azadirachta indica* (neem) and *Melia azedarach* (Chinaberry) at 8% concentration were tested against Ascochyta blight in field conditions. Although biological control agents are environmentally friendly, there is always a risk of adverse effects on non-target organisms or beneficial micro-organisms, which can lead to an imbalance in the ecosystem. Additionally, biological control agents are also costly as compared to chemical fungicides. Although these management practices can reduce the disease pressure, they do not provide complete disease control under field conditions. Due to this reason, genetic resistance is being explored as the most sustainable long-term strategy for controlling Ascochyta blight.

Genetic Resistance against Ascochyta blight complex in peas

Genetic resistance to Ascochyta blight in peas has been studied extensively at the level of the disease complex rather than at the level of individual pathogen species. There are very limited studies on resistance to specific pathogen species of Ascochyta blight complex. From different studies involving mixed epidemics of *D. pinodes*, *D. pinodella*, and *D. pisi*, resistance is described as partial, quantitative, and strongly shaped by host-pathogen-environment interaction (Bretag et al., 2006; Timmerman-Vaughan et al., 2002). The works by Fondevilla et al. (2005)

and Tiwari et al. (1997) showed that some pea lines had partial resistance, but no complete immunity was found. From their works, resistance to Ascochyta was described as quantitative i.e., controlled by multiple genes. Literature review suggests that there is limited information on the identification of QTLs associated with Ascochyta blight resistance in peas. In 2002, researchers aimed to understand the genetic basis of resistance to Ascochyta blight in peas by mapping quantitative trait loci (QTLs) associated with resistance. QTLs affecting Ascochyta blight were mapped in $F_{2:3}$ and $F_{2:4}$ families produced from a cross between resistant that resistance breeding line 3148-A88 and susceptible cultivar Rovar (Timmerman-Vaughan et al., 2002). Thirteen QTLs against seven linkages groups were identified. Among them, eight QTLs were reproducible across environments. They also identified QTLs that interact with phenology and escape, two important aspects of plant development. This work was among the first to demonstrate how complex and multi-locus resistance is in field conditions. Other studies also support multiple loci of small to moderate effect in Ascochyta blight disease complex infection (Jha et al., 2017; Prioul et al., 2004). However, these finding may be influenced by shifts in the dominant species of the Ascochyta blight complex across regions, years, and plant tissues and the resistance identified in mixed field epidemics does not necessarily represent resistance to each species in the complex. Thus, species specific studies are necessary to understand the nature of resistance for each species in Ascochyta blight complex.

Genetic Resistance against *D. pisi*

In research in 1950, the *D. pisi* resistant variety Austrian Winter was crossed with different susceptible varieties, and the progeny were tested for susceptibility to the disease. The results

indicated that resistance to the disease is controlled by three dominant genes, which must be present together for the resistance to be effective (Wark, 1950).In another research to study the resistance to *D. pisi*, research was conducted by making a cross between the susceptible variety, Thomas Laxton, and the resistant pea strain, Ottarva A-100. The findings show that resistance to the isolate employed is caused by duplicate dominant genes, in the ratio of 15 resistant to 1 susceptible, suggesting either of gene can provide resistance (Lyall et al., 1958). However, in another study where they crossed three susceptible cultivars with two resistant varieties, Rovar and Regis suggested *D. pisi* resistance to be governed by a single dominant gene Csizmadia (1995).

However, the resistance to *D. pisi* should not be viewed as a single uniform trait as the pathogen constitutes multiple races that differ in virulence, so the resistance of the pea genotype may depend upon the particular isolate or race selected (Ali et al., 1978; Darby et al., 1986; Wallen, 1957). Jones, (1927) first reported on physiological specialization in *D. pisi* and differences in pathogenicity between isolates of *D. pisi* from different countries. Based on the pathogenic behavior of *D. pisi* on peas, 31 different races of *D. pisi* have been isolated and identified so far Vladimirseva et al (1989). The existence of physiological races in *D. pisi* makes resistance breeding more difficult, as different races may respond differently to the resistance genes carried by the same pea variety. Resistance genes against *D. pisi* in pea have mostly been inferred from traditional inheritance studies rather than through cloning or detailed validation as species-specific loci

Resources for genetic resistance to Ascochyta Blight

The resistant resources available for Ascochyta blight include cultivars and breeding lines which have been reported as moderately resistant, wild and interspecific *Pisum* sources, plant introduction accessions and germplasm panels used for large-scale screening, and public diversity collections that can support further screening, and breeding.

Among cultivars, Radley remains as one of the sources for partial resistance to *D. pinodes*. Kraft (1998) reported that in further field screening of 2,936 accessions from the USDA Plant Introduction collection, five lines: PI 142442 (Peru), PI 381132 (Ethiopia), PI 142441 (Peru), PI 404221 (Russia), and PI 413691 (Hungary) matched Radley and were found resistant in Ireland and New Zealand sites. Additional cultivars: Yellowhead, Carneval, Miko, Danto, Majoret, Voyageur, and Montana were reported as relatively resistant in Ascochyta blight disease complex, in *D. pinodes* dominant studies (H. Wang et al., 2000). Breeding line 3148-A88 is one of the most significant Ascochyta blight complex resistance sources as it was the resistant parent in QTL mapping and validation studies that helped define the quantitative architecture of field resistance for the first time (Timmerman-Vaughan et al., 2002). Breeding lines 98107-62E, 05P770-BSR-705, 06P822-(F5)-BSR-6, ATC 866, and Dundale provided useful resistance against *D. pinodes* and *D. pinodella* (Tran et al., 2015). Screening of *Pisum* germplasm revealed that *P. fulvum* had some of the greatest levels of resistance to *D. pinodes*, followed by P. *sativum* subsp. *elatius* and subsp. *Syriacum* (Fondevilla et al., 2005). Another strong interspecific resource is *P. fulvum* accession P651, which served as the resistant parent in PR-19 recombinant inbred line population and contributed stable QTL for Ascochyta blight complex resistance (Jha et al., 2017). Another source, subsp. *syriacum accession* P665 has shown moderate resistance to

D. pinodes. The USDA Plant Introduction collection screened 558 field pea accessions against *D. pinodes* and confirmed that resistance is quantitative (Zhang et al., 2006). Recently, 1,276 *Pisum* accessions have been growing in Australia, to test the resistance against *D. pinodes* for GWAS and donor development (Lee et al., 2023).

Resources for resistance to D. pisi

The earliest result documented is from the 1940s in Georgia, USA, where 208 lines were screened against *D. pisi*, and the Austrian Winter Pea line was found to be the most resistant among them all (Stuckey, 1940). But the same outcome could not be seen when Weimer (1947) screened a large pea population against *D. pisi* over many years and could not find any resistance in cultivated peas, including the Austrian Winter Pea. Another variety, Ottawa A-100, showed a clear dominant inheritance when screened against *D. pisi* (Lyall, 1958). The most convincing recent evidence for species-specific resistance comes from purified inoculum screening where Fallon was found completely immune to *D. pisi* and another variety, Agassiz showed strong but incomplete resistance (Annan et al., 2023). Currently, Fallon is the only available variety showing complete immunity to *D. pisi*.

Genetic Mapping

Genetic Mapping is a process of determining the relative locations of genes on a chromosome. The mapping relies on the natural process of crossing over, also known as recombination, where homologous chromosomes exchange DNA during meiosis and it is based on the principles of inheritance as first described by Gregor Mendel in 1865 (Nimbalkar et al.,

2014). If two genes are close to each other on the same chromosome, they are more likely to stay together during crossing over and be inherited as a unit. The further apart two genes are, the more likely recombination will occur between them. Based on recombination frequency, genetic map measures the distance between genes and maps the gene to actual location in genome (B. D. Singh & Singh, 2015b). The distance between two genes is measured in units known as centimorgan or map units. Genetic or linkage maps are species specific and comprised of molecular markers or genes and the genetic distance between each marker.

In 1980, scientists were able to generate the first human genetic map based on the differences in DNA sequences as markers. Since then, molecular markers have been able to map the full genome of many organisms including plants and animals. The first genetic map of peas was published by T. H. Ellis et al (1992) organizing molecular markers in an organized linkage group. The accuracy and resolution of a genetic map are fundamentally dependent on the nature and size of the population used for mapping (Semagn et al., 2007). Therefore, careful selection of a mapping population is a critical requirement for in any genetic mapping.

Mapping population

The first step in genetic mapping is the development of a mapping population. A mapping population is the population developed by crossing two genetically diverse parents and then studying their progeny to find the gene of interest. The chosen parents must be genetically and phenotypically diverse to help find polymorphic markers for the traits of interest. When a mapping population is developed to find a specific trait, the parents must show a variation for the targeted trait, whether it is a phenotypic or genotypic variation.

The mapping population is categorized in different types according to the development stage, genetic stability and number of parents (Semagn et al., 2007). Temporary population include the F2 population by selfing F1 hybrids and backcross population derived by crossing the F1 hybrid to one of its parents. The temporary population are segregating and genetically unstable and cannot be maintained. Whereas the permanent population are the Recombinant Inbred line (RILs) which are a series of homozygous lines created by 6–10 generations of self-pollination and Doubled Haploids (DH) which are produced by doubling chromosomes of haploid cells (Semagn et al., 2007). The permanent population are developed through multiple generations of breeding to reach homozygosity, and then they can be share and multiplied endlessly. Biparent population are the ones developed from two genetically diverse parents, who are often homozygous and multiparent population involve more than two parents to increase mapping resolution and genetic diversity.

Regardless of the mapping population type selected, the ability to detect recombination events within the population depends entirely on the availability of informative molecular markers. The choice of marker plays an important role in determining the efficiency and resolution of the genetic map constructed.

MATERIALS AND METHODS

Plant material

Mapping populations and growth conditions

The mapping populations in this study were developed from a cross between two pea varieties: Fallon and Stirling. Fallon is resistant to *D. pisi* and was used as a male parent. Stirling is susceptible to *D. pisi* and was the female parent in the cross due to its bigger flower size and high seed set. The cross was done in a greenhouse of the Plant Growth Center (PGC) of Montana State University (MSU) by Dr. Kevin McPhee from the pulse breeding program.

F1 plants were advanced to F2 and further $F_{2:3}$ in a greenhouse with one plant/pot. To increase seeds for phenotyping infection types against *D. pisi*, seeds of each $F_{2:3}$ family were grown in rows as plots in the fields of MSU research Post Farm to obtain $F_{2:4}$ population. Some of the $F_{2:4}$ families were further advanced to $F_{2:5}$ families as a mapping population.

For progeny tests, 20 seeds from each family of $F_{2:4}$ or $F_{2:5}$ generation were grown with five plants/pot. The seeds were sown in 6-inch pots with soil containing a mixture of soil and peat Sunshine Mix (Sun Gro Horticulture, Agawam, MA, USA) at a ratio of 1:1. The greenhouse was set with day/night temperatures of 30.2/18 °C, 22% humidity, and 16 h of light. The progeny tests, pathogen inoculation, and disease screening were performed on the 238 $F_{2:4}$ plants and 262 $F_{2:5}$ plants.

Pathogens and growth conditions

To study the genetic resistance of *D. pisi* in Fallon, two isolates of *D. pisi* species were used for the study. Isolate '22-P2033'was isolated from Ascochyta Blight contaminated seed submitted by growers to the Regional Pulse Crop Diagnostic Laboratory (RPCDL) at MSU. Isolate '10728' was provided by Dr. Frankie Crutcher in the Eastern Agricultural Research Center, MSU. Both isolates were purified and confirmed as pure cultures of the species by PCR using species-specific primers and sequencing (Owati et al., 2019).

The isolates were cultured on plates with pea meal agar (PMA) (Fig. 2A), consisting of 25 g of blended dry pea and 15 g of Agar in 1 L of double-distilled water (ddH2O), at room temperature (E. N. Annan et al., 2023).

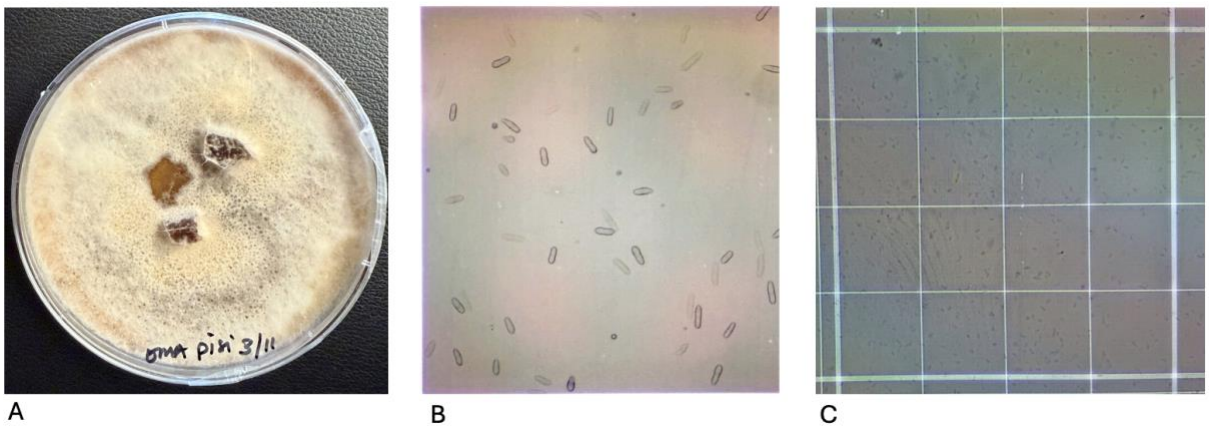


Figure 2: *D. pisi* isolate '10728' (A) D. pisi in a pea meal agar plate. (B) *D. pisi* spores under a compound microscope at 100X resolution (C) *D. pisi* spores in a hemacytometer under a compound microscope at 40X resolution.

Inoculum preparation

The single-species *D. pisi* inoculum was prepared by flooding the surface of the cultured fungus in a PMA plate with sterile ddH2O and gently scraping the flooded culture surface with a

pipette until all the conidia were dislodged into the water. The liquid was then filtered through one layer of cheesecloth and used as a stock for the inoculum. Then, 10 µl stock solution was pipetted onto a hemacytometer, and the spore density was counted under a light microscope (Fig. 2B and 2C). The liquid inoculum was prepared by diluting a calculated amount to achieve a spore density of 10^5 spores/ml. Tween 20 was added in the ratio of two drops per 200 ml inoculum (E. N. Annan et al., 2023).

Pathogen inoculation

The plants were inoculated at the four-leaf stage via sprayed inoculation to cover every part of the plants (Fig. 3A). The inoculated plants were kept in a dew chamber (Fig. 3B) with an air temperature of 22 °C for 24 hours in dark and then transferred to a plastic tent inside the greenhouse (Fig. 3C). A high humid condition was maintained inside the tent by misting the plants by humidifiers for at least 2 to 3 days after pathogen inoculation.

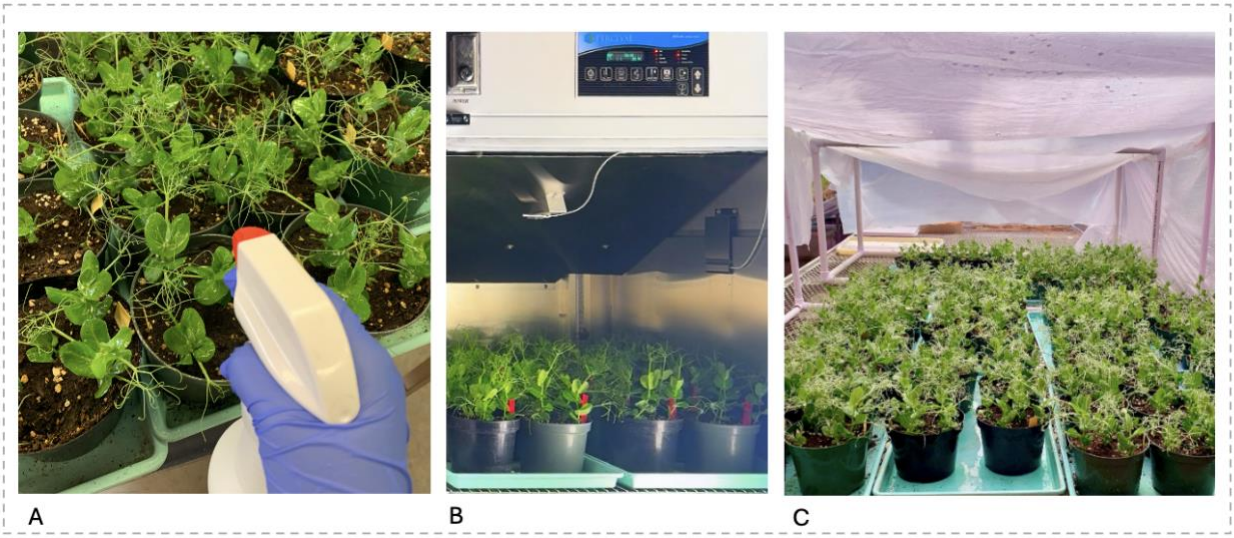


Figure 3: Steps of pathogen Inoculation. (A) Spraying *D. pisi* inoculum onto plants. (B) Incubating the inoculated plants in a dew chamber for 24 hours to maintain optimum humidity for fungal pathogen growth. (C) Storing the inoculated plants in a plastic tent in the greenhouse to maintain optimal humidity.

Pathogen inoculation

Disease scoring was done based on the phenotype of the susceptible control plants. Under controlled conditions, 14 days post-inoculation, a visual disease symptom was seen on the susceptible plants, whereas the resistant plants showed no symptoms of disease.

For progeny testing, when all 20 plants in a family showed a disease symptom, the family was scored as a homogeneous susceptible family; if all 20 plants showed a resistant phenotype, it was scored as a homogeneous resistant family. The families in which the 20 plants showed both resistant and susceptible phenotypes were counted as heterozygous families.

One leaf from every 20 plants in a homogeneous susceptible family was collected and pooled to obtain 20 leaves per family. Similar sampling was done for homogeneous resistant

families. Collected leaves were immediately frozen in liquid nitrogen and transferred to -80 ºC for storage.

DNA extraction

DNAs were extracted by using a QIAGEN DNeasy Plant Pro Kit (QIAGEN, Valencia, CA, USA). The extraction protocol was followed according to the manufacturer's instructions. Concentrations of DNAs were measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). The integrity of the DNA was assessed by electrophoresis on a 1% agarose gel with GelRed (Bio-Rad, Hercules, CA, USA). Each sample was loaded with 2 µl of DNA and 3 µl of loading buffer (98% formamide, 10 mM EDTA, 0.25% bromophenol blue, and 0.25% xylene cyanole).

Bulk preparation

The resistant bulk was prepared by mixing an equal amount of DNA from 34 homogeneous resistant families. Similarly, the susceptible bulk was prepared by mixing equal amounts of DNA from 34 homogeneous susceptible families.

Microsatellite markers assays

To map the resistance genes to pea chromosomes, 42 microsatellite markers were selected, six from each arm of the pea chromosome, based on the markers designed by Loridon et al. (2005). Primers of the markers were obtained from the pulse breeding program of Montana State University. PCR amplification was performed in a 20 µL reaction volume. Thermal cycling conditions consisted of an initial denaturation at 95 °C for 3 minutes, followed by 39 cycles of

denaturation at 95 °C for 10 seconds, annealing for 45 seconds (annealing temperature was adjusted based on primer melting temperature), and extension at 72 °C for 30 seconds. A final extension was carried out at 72 °C for 10 min.

The gene linkage map construction

The linkage map of the resistance gene was based on recombination frequencies < 50%, calculated using the following formula(Lobo et al., 2008).

$$\text{Recombination frequency} = \frac{\text{Number of Recombinations}}{\text{Total number of Offspring} \times 2} \times 100$$

Whole genome sequencing

Four samples of genomic DNAs, including the resistant parent (Fallon), the susceptible parent (Stirling), the resistant bulk, and the susceptible bulk, were sequenced by Azenta (Burlington, Massachusetts) using Illumina NovaSeq technology for whole-genome sequencing. Sequence alignment and variant calling. Raw sequencing reads were processed through a standard bioinformatics pipeline for variant identification. Reads were aligned to the reference genome '*Pisum sativum* v1a genome' using BWA-MEM and then followed by variant calling using VarScan2. The built-in quality control parameters of VarScan2 were used to filter the variants where minimum read depth ≥10×, minimum base quality ≥15, minimum mapping quality ≥20, minimum variant frequency threshold ≥0.01, and a statistical significance filter with P-value ≤0.05. Variants in low-complexity and repetitive regions were removed as additional post-calling filters. Variants designated as "PASS" in the filter field were the only ones kept for

further examination. Variants were classified as single-nucleotide polymorphisms (SNPs) or insertion/deletion events (indels) based on the length difference between reference and alternate alleles.

Filtering for resistant-associated variants. Variant data from the four samples were analyzed to identify genetic markers associated with resistance phenotypes. Custom Python scripts were developed to parse VCF files and extract relevant variant information, including reference allele counts (RD), alternate allele counts (AD), total depth (DP), allele frequencies (FREQ), and statistical significance values (PVAL). The SNPs common to Fallon and R-bulk and different from Stirling and S-bulk were filtered. The genotypes of the parents and bulks were called when the allele frequency ≥ 0.85. For filtering criteria, the read depth was a minimum of 20 at each variant position; variants with SNP index ≥ 0.3 were considered potential resistant-associated variants, and those with SNP index ≥ 0.5 were considered strong resistant-associated variants. Similarly, variants between two bulks with an allele frequency difference $\geq 50\%$ were considered as strongly resistant-associated variants. Every variant was also evaluated for statistical significance, a P-value below 0.05 was required for the variants to be retained.

A sliding window approach at 1 Mb window size and 1 kb step size was also used to look at regions of resistance association across the genome and confirm if the variants selected by previous filtering criteria fall in the resistance peaks shown by the sliding windows. The additional SNPs in the resistance peaks were also selected for further filtering. Finally, SNPs in a gene or 2000 bp upstream of a gene were selected as resistance associated variants.

Designing primers for selected resistance-associated variants. To validate the identified variants, primers were designed to target the genomic regions surrounding each strong resistance-associated variant (Appendices table A1). Flanking sequences were extracted from the reference genome, and primer design parameters included optimal melting temperatures (55-65°C), GC content (50-60%), and specificity to avoid off-target amplification with the temperature difference between forward and reverse primer maintained at less than 5°C. The quality of primers was checked using a tool from the Integrated DNA Technologies website (Integrated DNA Technologies, 2026). Primer specificity was checked using Ensembl (Ensembl Plants, 2026).

Validating the resistance-associated markers. Newly designed primers were validated on the two parental lines to confirm the SNPs revealed by whole-genome sequencing. PCR products of 25 reactions of Fallon were pooled, and the same pooling for Stirling, and were sequenced by using the Oxford Nanopore Technology (ONT) sequencing service of Plasmidsaurus (city, state). Sequencing results were analyzed to confirm the presence of the SNPs. The sequencing reads were aligned to the reference sequences using minimap2 (v2.30) with the ONT long-read preset. Aligned reads were sorted and indexed using SAMtools (v1.22.1). Sequencing depth across all loci ranged from 88–340x, confirming sufficient coverage for reliable variant detection. At each position, the number of reads supporting each base pair was counted, and genotype was called for both Fallon and Stirling. MAFFT (v7.525) was used to create multiple sequence alignments of the reference sequence, the Fallon consensus sequence, and the Stirling consensus sequence, which were then displayed in AliView and IGV.

Confirming the resistance-associated markers in individual families. The markers that showed clear differences in allele frequencies and distinct genotype calls between the resistant and susceptible parents were selected for further validation with PCR amplification on individual resistant and susceptible plants and sequencing the amplified products and assessing whether SNP genotypes are co-segregated with the phenotypes as expected

RESULT

Genetic analysis of resistance to D. pisi in Fallon

To determine the genotype of the resistance gene in the families of the mapping populations, we conducted progeny testing on 501 families, comprising 238 $F_{2:4}$ and 262 $F_{2:5}$, with two isolates. The $F_{2:4}$ population was inoculated with *D. pisi* isolate '22-P2033' and the $F_{2:5}$ population was inoculated with *D. pisi* isolate '10728'.

The infection types of the two parental lines were similar for the two isolates. Fallon was immune without any visible symptoms (Fig. 4A), and Stirling showed clear blight spots (Fig. 4B). Infection types of 20 plants of each family were clear and distinguishable as shown in Figure 4. All 20 plants showed resistant phenotypes (Fig. 5A), this family was scored as a homogeneous resistant family. When the 20 plants of a family showed both resistant and susceptible phenotypes, the family was scored as a heterozygous family (Fig. 5B), and when all the 20 plants of a family showed susceptible phenotypes, the family was scored as a homogeneous susceptible family (Fig. 5C).

After disease screening of isolate '22-P2033' in the $F_{2:4}$ population, 12 out of 238 (~5.04%) families were identified as homogeneous resistant, and the remaining families were either homogeneous (34 families) or heterozygous susceptible (Table 1). The resistant: susceptible genotype ratio was close to 1:15, consistent with a model of two independent genes segregating, in which two recessive genes on different chromosomes confer resistance. The result was confirmed by chi-square (χ^2) analysis, where the calculated chi-square value was 0.60 with 1 degree of freedom, which is lower than the critical value of 3.84 at $p = 0.05$.

However, disease screening in the $F_{2:5}$ population using isolate '10728' revealed a different result. Among 262 families, 74 (~28.24%) were homogeneous resistant, and the remaining families were either homogeneous (35 families) or heterozygous susceptible (Table 1). The resistant: susceptible genotype ratio was 1:3, which fits the model in which resistance is controlled by a single recessive gene. The result was confirmed by chi-square (χ^2) analysis, where the calculated chi-square value was 1.74 with 1 degree of freedom, which is lower than the critical value of 3.84 at $p = 0.05$.

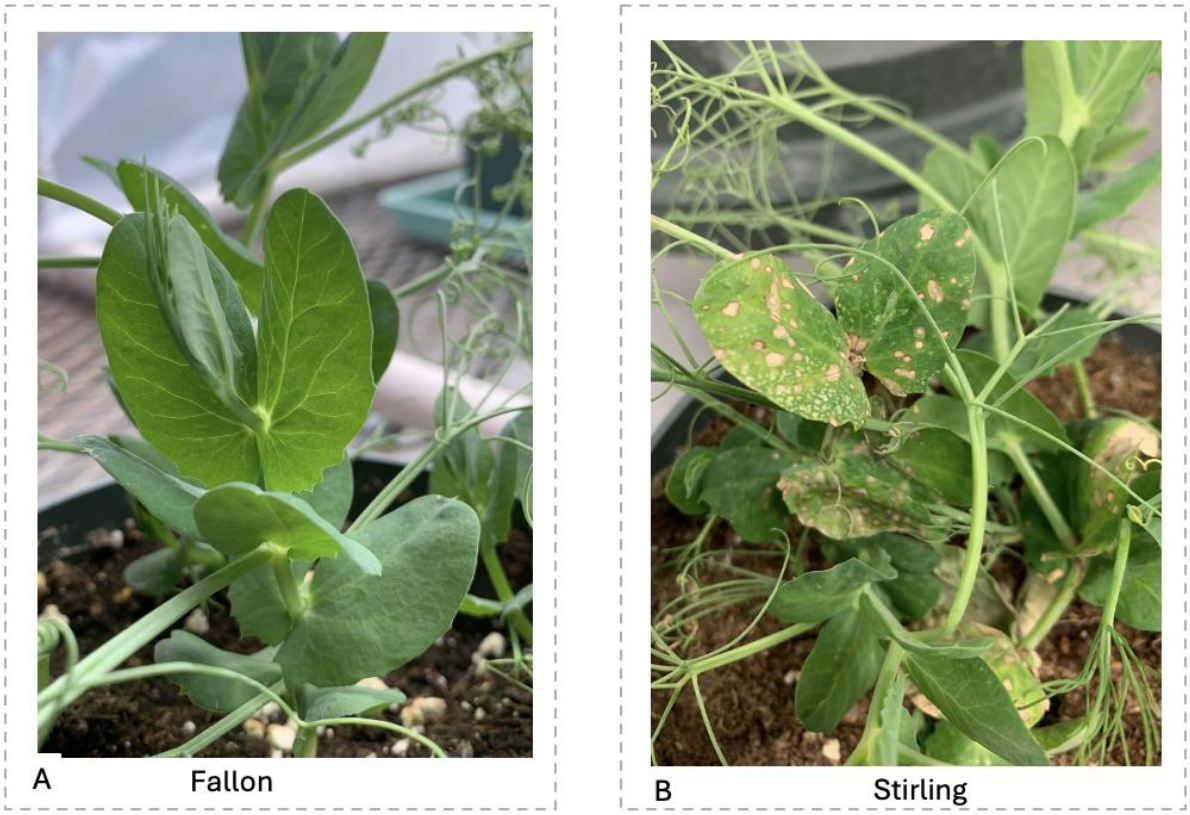


Figure 4: Pea plants 14 days post inoculation with *D. pisi* isolate '22-P2033'. (A) Fallon is the resistant parent. (B) Stirling is the susceptible parent.

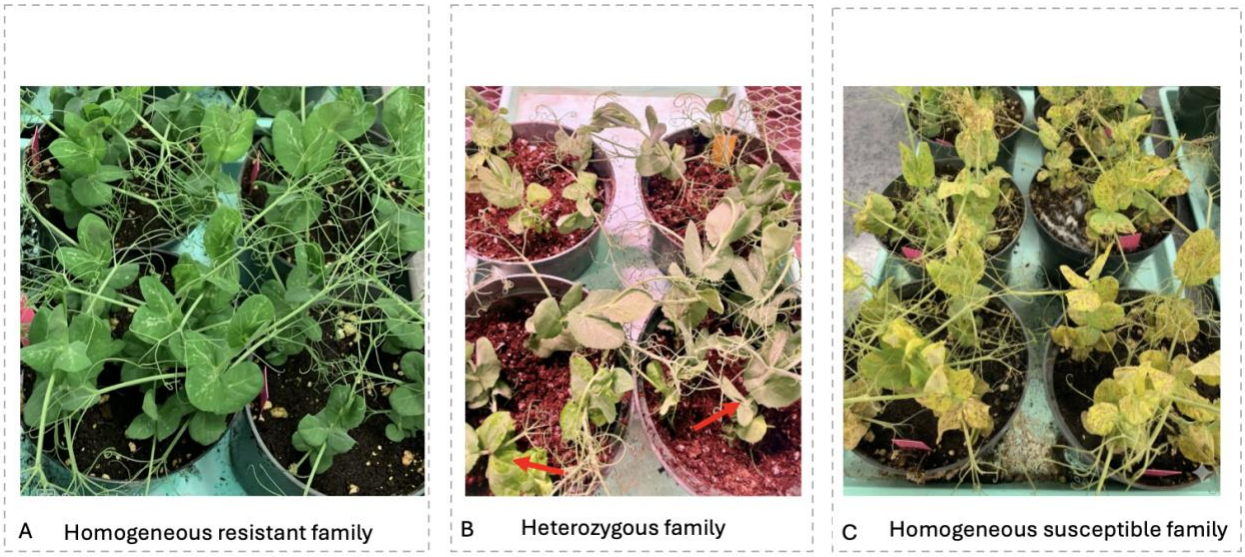


Figure 5: Pea plants 15 days post inoculation with *D. pisi* isolate '22-P2033'. (A) A homogeneous resistant family where all plants show a resistant phenotype against *D. pisi*. (B) A heterozygous family where plants show both resistant and susceptible phenotypes against *D. pisi*. (C) A homogeneous susceptible family where all plants show susceptible phenotype against *D. pisi.*

Table 1: Segregation of resistant and susceptible phenotypes in $F_{2:4}$ families against *D. pisi* isolate '22-P2033' and in $F_{2:5}$ family against *D. pisi* isolate '10728'

	Isolate '22-P2033'	Isolate '10728'
Total screened families	238	262
Homogeneous susceptible families	34	35
Homogeneous resistant families	12	74
Heterozygous families	192	153

Molecular mapping of the resistant genes to chromosomes

Since the number of resistance genes in Fallon against different *D. pisi* isolates varied, molecular mapping of the genes conferring resistance to isolates '22-P2033' and '10728' was performed separately in $F_{2:4}$ and $F_{2:5}$ using their respective isolates.

Polymorphism between the parents and the bulks

To cost-effectively map the molecular markers linked to the resistance genes, we used bulk-segregant analysis. Thirty-four homozygous families, resistant or susceptible, were pooled to produce an R bulk or an S bulk, respectively (for details, see M&M). From the available genetic maps and microsatellite markers (Loridon et al., 2005), 42 markers were selected, three per chromosome arm, to represent all the fourteen arms of the seven pea chromosomes. The selected markers were first tested in the two parental lines to check whether polymorphisms were observed. Six markers: D21, AB180, AD51, AA475, AA387, and Fa LL R2 showed polymorphism between the two parents. Then, the polymorphic markers were tested on the R and S bulks. Two markers, AD180 and AD51, showed the same bands between the resistant parent Fallon and the R-bulk, but differed between the susceptible parent Stirling and the S-bulk. The gel electrophoresis images of the two markers, AD180 and AD51, are shown in Figure 6. The polymorphism revealed by AD180 consists of two bands indicated by arrows, a co-dominant marker (Fig. 6A). The polymorphism revealed by the marker AD51 is a 500-bp fragment present in the resistant parent and the R bulk but absent in the susceptible parent and reduced in the S bulk (Fig. 6B).

The gene linkage map construction

The initial linkage maps were constructed based on recombination frequencies from the individual families used to generate the R and S bulks.

Since the number of resistance genes against the two *D. pisi* isolates in Fallon was different, therefore the two resistance genes against isolate '22-P2033', designated as *R1-33* and *R2-33*, were mapped in the $F_{2:4}$ population using the 46 homozygous families (12 resistant and 34 susceptible). The resistance genotypes of the 46 families were segregated with AB20 (Fig. 7A) at a 28% segregation ratio (28 cM) and with A5 (Fig. 7A) at a 31% segregation ratio (31 cM). The two markers are mapped on chromosome I in the linkage map by Loridon (2005), so eight available markers surrounding the AB20 locus were also selected from chromosome I, and five of them showed polymorphism between the parental lines and bulks and were mapped 31 cM (A5), 35 cM (AA335 and AD160), 44 cM (AA467), and 48 cM (AA103) away from the *R1-33* gene (Fig. 7A).

The resistance genotypes also segregated with AD51 (Fig. 7B) at a 17% segregation ratio (17 cM), and with AD180 (Fig. 7B) at a 24% segregation ratio (24 cM). The marker AD51 is mapped to chromosomes I and V, and AD180 is mapped on chromosome V by Loridon (2005). In the mapping population of Fallon and Stirling, AD51 is mapped on chromosome V along with AD180. The molecular mapping suggests that one resistance gene (*R1-33*) is located on the same chromosome I as AB20 and A5 and the other resistance gene (*R2-33*) is linked with AD51 and AD 180 on chromosome V.

The resistance gene against the *D. pisi* isolate '10728', designated as *R-28*, was mapped in the $F_{2:5}$ population using 74 homogeneous resistant families. AD51 was 24.71 cM away and

AD180 was 25.86 cM away from *R-28* gene, respectively. The two markers, AD180 and AD51, were 19.54 cM away linked on the same chromosome (Fig. 7C).

Our results suggested that one of the resistance genes against both isolates was mapped to the same chromosome V, linked to AD180 and AD51. The other resistance gene required for resistance against isolate '22-P2033' was mapped to chromosome I (Fig. 7).

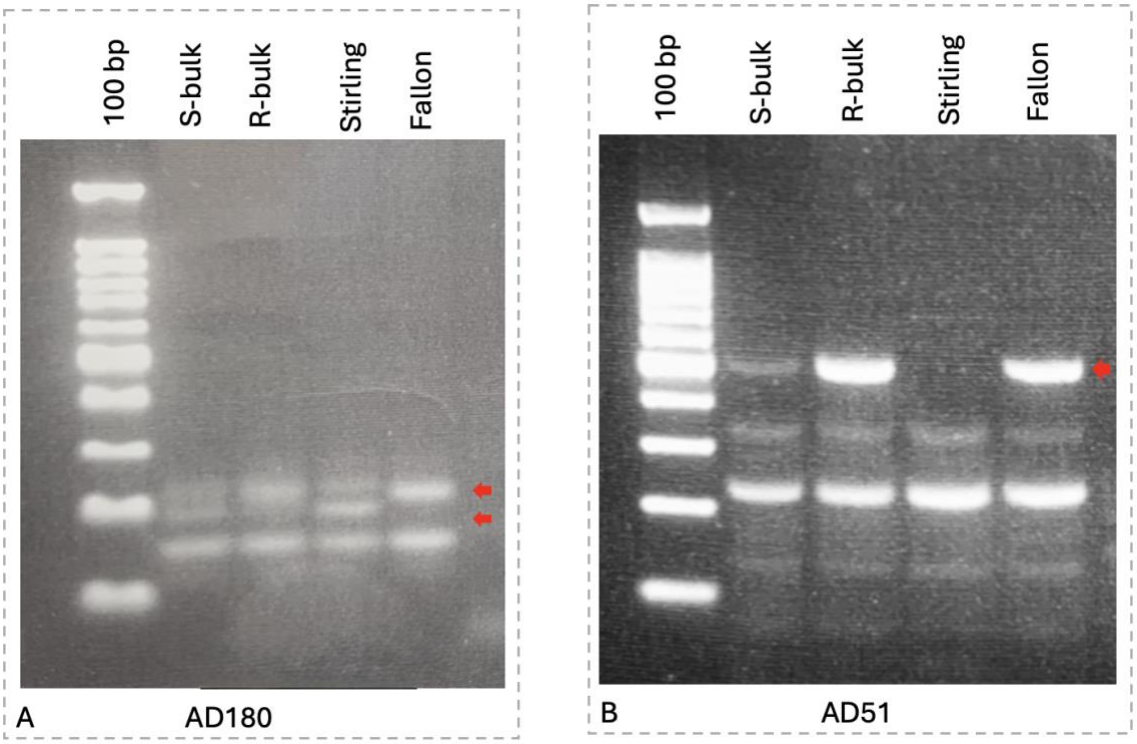


Figure 6: Polymorphism detected by microsatellite markers between resistant parent, susceptible parent, R-bulk and S-bulk. (A) Marker AD180 (B) Marker AD51

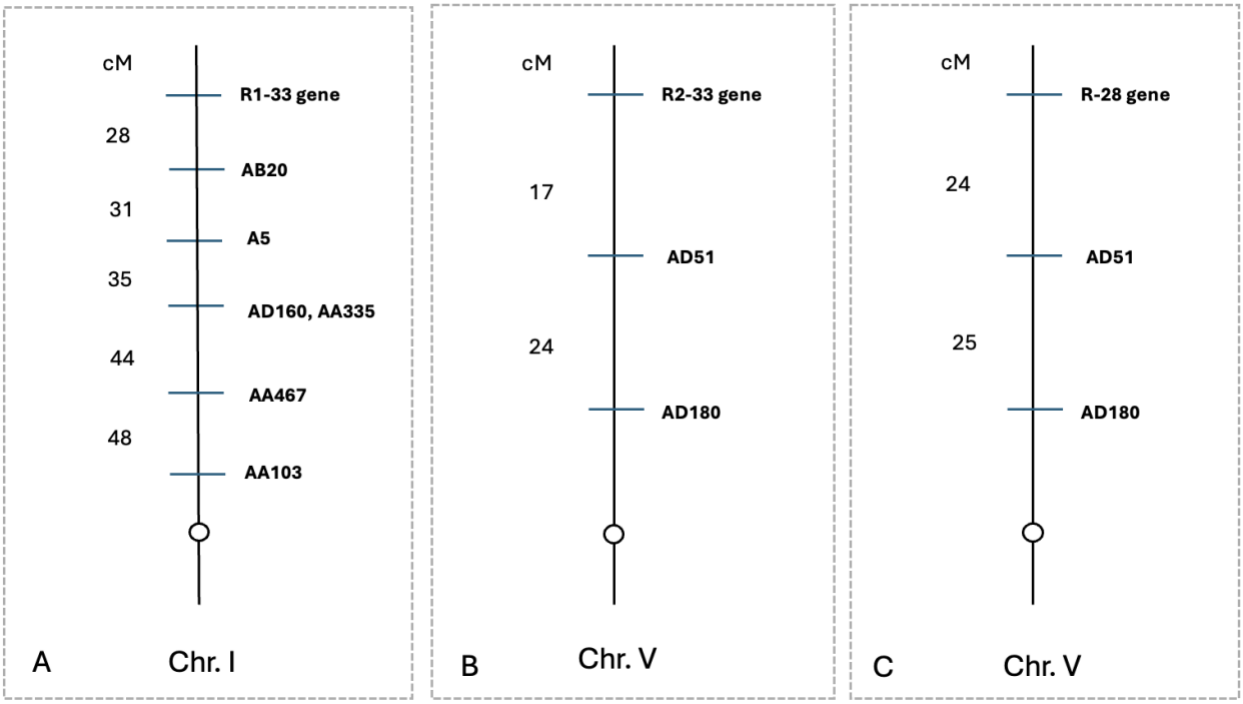


Figure 7: Linkage maps of the genes conferring resistance against *D. pisi*. (A) The R1-33 against *D. pisi* isolate '22-P2033' in chromosome I. B) The R2-33 gene against *D. pisi* isolate '22-P2033' in chromosome V. C) The R-28 gene against *D. pisi* isolate '10728' in chromosome V.

Whole genome sequencing

Based on molecular mapping using available microsatellite markers, tightly linked markers for selecting the gene of resistance for both the *D. pisi* isolates, isolate '22-P2033' and isolate '10728' have not been identified. Therefore, to capture additional polymorphisms between the parental lines and the resistant and susceptible bulks, whole-genome sequencing (WGS) was performed.

Whole genome sequencing generated high-quality variants across 9,746 chromosomes/scaffolds. A total of 1,797,679 variants were identified across four samples, comprising 1,631,943 SNPs and 165,736 indels, among which 489,921 variants were identified

in Fallon, 526,086 variants were identified in Stirling, 371,030 variants were identified in R-bulk, and 410,642 variants were identified in S-bulk. All variants passed 100.0% quality filters. Comparative analysis between resistant and susceptible samples revealed 235,018 shared variant positions and 513,293 variants unique to resistant samples and 563,821 variants unique to susceptible samples as compared to the reference genome.

The variants common to Fallon and R-bulk and different from Stirling and S-bulk were filtered.

The allele frequency was calculated from the formula:

Allele Frequency = Alternate reads / (Reference reads + Alternate reads) × 100.

After the initial filtering, SNPs in the gene and in around 2000 bp proximity of genes were selected. Twenty-five positions were selected as highly confident SNPs for resistance, and primers were designed from the flanking sequences of the SNP positions (Supplement table 1). The selected variants (in red color) were included from chromosomes I, II, III, V, VI, VII. There were 16 SNPs in a gene and nine SNPs in around 2000 bp upstream region of the gene (Table 2). The chromosomal positions, proximity from the gene and gene annotations of the SNPs are presented in Table 2.

Table 2: High confident SNPs from the whole genome sequencing variants results, along with the chromosome number, SNP position, proximity from a known gene with gene annotation. Red color indicates the SNPs selected for further validation.

Chromosome	Position	Distance from gene	Gene name	Annotation
VI	278652394	In gene	Psat6g141200	
VII	18415914	In gene	Psat7g011720	Catalytic activity, hydrolase activity, carbohydrate metabolic processes
VII	18416152	In gene	Psat7g011720	Catalytic activity, hydrolase activity, carbohydrate metabolic processes
VII	145325576	In gene	Psat7g087120	
VII	225565863	In gene	Psat7g134560	
VII	225565871	In gene	Psat7g134560	
VII	245767940	In gene	Psat7g134560	
V	80936153	In gene	Psat5g044640	Transporter in cell membrane
V	111682088	In gene	Psat5g062040	Oligopeptide transporter
V	383599011	713 bp	Psat5g192720	Metal ion homeostasis
V	383599025	727 bp	Psat5g192720	
V	436271050	In gene	Psat5g213160	Proteasome-mediated ubiquitin-dependent protein catabolic processes**.** hormone signaling in flowering time and stress tolerance**.**
V	436271052	In gene	Psat5g213160	

Table 2 continued

Chromosome	Position	Distance from gene	Gene name	Annotation
V	436271896	In gene	Psat5g213160	
V	450850110	240 bp	Psat5g225080	Metabolic processes, including the synthesis of plant hormones, fatty acids, and defensive compounds (secondary metabolites)
V	548974980	In gene	Psat5g283200	Transmembrane transporter activity
V	337117658	1.8 kb	Psat5g182360	Biosynthetic processes for plant hormones, fatty acids and defensive secondary metabolites.
III	192934874	In gene	Psat3g095480	Cell wall modification, defense to stress
VI	295348273	2 kb	Psat6g151400	Cell wall biogenesis and polysac charide metabolic processes.
II	105233936	In gene	Psat2g060440	Signal transduction and the regulation of plant development.
III	192934885	In gene	Psat3g095480	Cell wall modification, defense to stress
V	134307453	3 kb	Psat5g074120	Proteasome-mediated ubiquitin-dependent protein catabolic processes.
VII	318216082	617 bp	Psat7g165840	Proteasome-mediated ubiquitin-dependent protein catabolic processes.
VII	94901789	1.5 kb	Psat7g056160	Proteasome-mediated ubiquitin-dependent

Table 2 continued

				protein catabolic processes.
Chromosome	Position	Distance from gene	Gene name	Annotation
I	61885991	1.8 kb	Psat1g040160	Proteasome-mediated ubiquitin-dependent protein catabolic processes.

(Kreplak et al., 2019; *PulseDB*, 2026; *URGI*, 2026)

Validating the resistant associated markers

The newly designed primers were validated on the two parental lines to confirm the SNPs revealed by whole-genome sequencing. PCR amplification and sequencing by Oxford Nanopore Technology (ONT) sequencing service were performed on Fallon and Stirling.

PCR amplification

PCR amplification was performed on the resistant and the susceptible parents. All primers showed good amplification producing amplicon sizes ranging from 100 to 500 bp. The gel electrophoresis images for three markers: C7SNP1, C5SNP7 and Ch9779 are presented in Figure 8. Markers C7SNP1 and Ch9779 show bands at 200 bp (Fig. 8A & 8C) and marker C5SNP7 shows a band at 500 bp (Fig. 8B). As the primers were designed for SNPs, no polymorphism in band size was detected between the resistant and susceptible parents.

Oxford Nanopore Technology (ONT) sequencing

PCR products of 25 reactions of each Fallon and Stirling were pooled and sequenced by Oxford Nanopore Technology (ONT) sequencing (Plasmidsaurus, Louisville, Kentucy). The sequencing reads were analyzed for the resistant and susceptible parents to confirm the presence of the selected SNPs. Three markers, C7SNP1, C5SNP7 and Ch9779 exhibited differences in allele frequencies and distinct genotype calls between the resistant and susceptible parents. At the selected positions, for markers C7SNP1 and C5SNP7, Fallon represented homozygous alternate genotype and Stirling represented heterozygous genotype. However, for marker Ch9779, Fallon represented homozygous reference genotype and Stirling represented homozygous alternate genotype (Table 3). The three markers were selected for further validation.

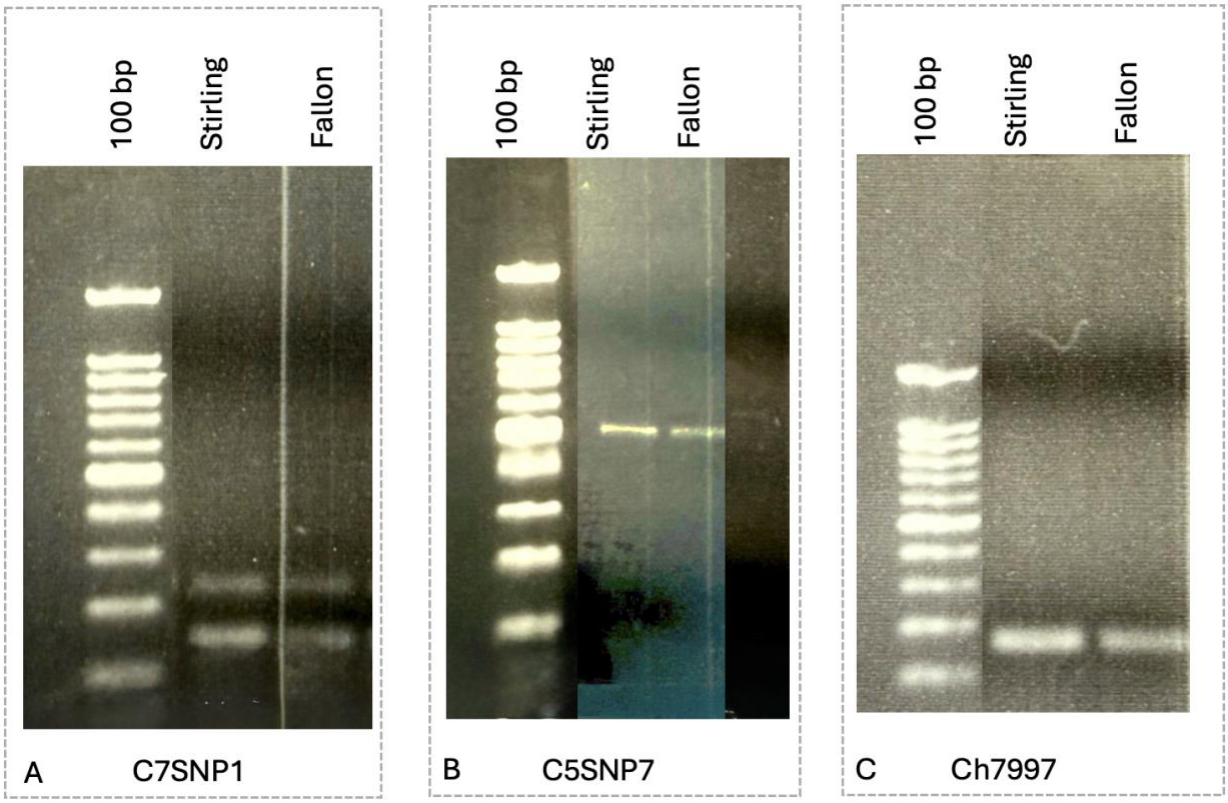


Figure 8: Polymorphism detected by markers between resistant parent and susceptible parent. (A) Marker C7SNP1 (B) Marker C5SNP7 (C) Marker Ch7997

Table 3: Selected SNPs with distinct genotype between the parents from ONT sequencing

Chromosome	**Position**	**Fallon**					**Stirling**				
		DP	Ref	Alt	AF	G	DP	Ref	Alt	AF	G
VII (C7SNP1)	18415914	321	15	305	0.95	Hom_Alt	221	55	163	0.72	Het
V (C5SNP7)	436271896	283	29	254	0.89	Hom_Alt	301	137	163	0.54	Het
VII (Ch7997)	318216082	102	102	0	100	Hom_Ref	93	0	93	100	Hom_Alt

Confirming the resistance-associated markers in individual families

Further validation by testing the three markers Ch7997, C7SNP1, and C5SNP7 in individual resistant and susceptible families is needed to confirm their association with the resistance genes.

DISCUSSION

Necrotrophic plant pathogens secrete toxins, proteins, cell wall-degrading enzymes (CWDEs), or secondary metabolites to break down the plant cell walls and damage the host cells. These pathogens kill their hosts and obtain nutrients from dead tissues. Plants resist necrotrophs by building stronger cell walls, detoxifying toxins, activating chemical defenses, or modifying their phytotoxin receptors (Kan, 2006). Active defense against necrotic pathogens is most commonly associated with dominant resistance, in which dominant resistance genes enable the host to recognize the pathogen and activate defense responses. In contrast, passive resistance is often linked with recessive resistance, which results from the loss or alteration of host genes needed by the pathogen for infection. In such cases, the pathogen is unable to utilize essential host components required for penetration, colonization, or suppression of plant defenses.

In this study, the resistance to isolate '22-P2033' is conferred by two homozygous recessive genes, and resistance to isolate '10728' is conferred by a single recessive gene in Fallon. The results suggest that the two *D. pisi* isolates may differ in the host factors they require for infection. For isolate '22-P2033', as two recessive genes are required for resistance, the pathogen has successful infection depending on two host receptors and the resistance is expressed only when both receptors are insensitive to the phytotoxin. It is likely that the isolate '22-P2033' secretes two different phytotoxins as two host receptors are needed for resistance. As for isolate '10728', one recessive gene is required for resistance, and the resistance occurs with loss or modification of one host receptor. So, it is likely that the isolate '10728', secretes only one type of phytotoxin. From an evolutionary perspective, the isolate '22-P2033' must have evolved from

its previous isolate to produce two distinct phytotoxins and to require modification of two host factors for successful resistance. The differences between the two isolates are consistent with the possibility that the two isolates represent different races.

D. pisi is divided into different races based on its pathogenicity. Jones (1927) published the first literature about the physiological races of *D. pisi* and so far, 31 different races have been isolated and identified (Vladimirseva et al., 1989). Studies suggest that phytotoxin, 'ascochitine', is secreted by *D. pisi* to cause infection in the host (Kaur & Deshpande, 1980; Lepoivre, 1982). Although there is no published evidence about race-specific differences in the quantity or intensity of ascochitine production in different races of *D. pisi*, variation in ascochitine production may contribute to pathogenic differences among races. Other studies have suggested that *D. pisi* isolates may differ in pathogenicity because they may differ in aggressiveness or virulence level, interaction with host (Darby et al., 1986) and response to host chemical defense pisatin (Harrower, 1973). In a recent research, Liu et al. (2023), concluded that during infection, *D. pisi* likely uses a number of virulence factors, such as secreted proteins, secondary metabolites, and CAZymes that aid in the degradation of host tissue. The different races of *D. pisi* may differ in the enzymes, proteins, and secondary metabolites, along with ascochitine, to trigger different resistance responses in the host.

With the widespread of more aggressive species, *D. pinodes* and *D. pinodella*, the literature is much richer for them as compared to specific studies on *D pisi*. The resistant genes *for D. pisi* have not been mapped in detail in any host (Jha et al., 2022; Timmerman-Vaughan et al., 2002). Studies till now have shown that resistance to *D. pisi* is conferred by a combination of three

dominant genes where the expression of all three genes is required for resistance, by duplicate dominant genes where dominance of either gene can confer resistance and by a single recessive gene (Lyall, 1958; Wark, 1950). However, the results are from studies on particular races of *D. pisi* and mapping population, thus, the results differ from this research. Resistance genes against *D. pisi* in pea have mostly been inferred from traditional inheritance studies rather than through cloning or detailed validation as species-specific loci. Further investigation is needed to better understand the race specific pathogenicity and race structure of *D. pisi* and its resistance in host plants.

After confirming the number of resistant genes against *D. pisi* in Fallon for two isolates '22-P2033' and '10728', the resistant genes were mapped to pea chromosomes by microsatellite markers developed by Loridon et al. (2005). The microsatellite markers were first developed and screened for polymorphism across two pea genotypes and then were mapped using three recombinant inbred line populations derived from the crosses Térésè × K586, Champagne × Térésè, and Shawnee × Bohatyr to assign the markers to linkage group.

The resistance genes R2-33 and R-28 against the two isolates are mapped to the same chromosome V, at different genetic distances from the marker AD51. However, since the maps were constructed using populations of different sizes, this doesn't rule out the possibility that the two genes are alleles or a common host receptor shared by both isolates. Based on microsatellite markers, no marker tightly linked to the resistance gene for either was identified. Therefore, to capture a higher density of polymorphisms between the parental lines and the resistant and susceptible bulks, whole-genome sequencing was performed. The whole genome sequencing was

performed for Fallon, Stirling, R-bulk and S-bulk. An important limitation of our study is the use of two *D. pisi* isolates during the phenotypic screening. As the isolates belong to two different races and trigger different genes and resistance responses in hosts, the resulting resistant and susceptible bulks may comprise a mixture of genotypes governed by multiple genetic mechanisms. As a consequence, allele-frequency variation between bulks may have been diluted, making it harder to identify clear, robust genomic signals associated with specific resistance loci. Thus, the sequencing graph also showed moderate-effect regions, rather than the expected single, large peak in bulk.

Despite the complexity introduced by multiple isolates, allele depth was used to filter variants that differed from Stirling and the susceptible bulk but were consistent between Fallon and the resistant bulk. The selected variants were also filtered on the basis of their proximity to the gene. The selected variants were from chromosomes I, II, III, V, VI, VII. There were 16 SNPs in genes and nine SNPs in around 2000 bp upstream region of the gene. After validating the resistant-associated markers with PCR amplification on parental lines and ONT sequencing service, three markers: Ch7997, C7SNP1, and C5SNP7 from chromosome V and two from chromosome VII were selected based on the results of Oxford Nanopore sequencing technology. Further validation by testing the three markers on individual resistant and susceptible families is needed to confirm the resistance-associated markers.

To precisely map race-specific resistance genes and gain a better knowledge of the genetic basis of resistance, future research should concentrate on assessing resistance using individual, well-characterized isolates.

CONCLUSION

The study successfully determined the genetic basis of resistance against two *D. pisi* isolates in Fallon by two recessive genes and established their chromosomal positions.

Genetic analysis of 238 $F_{2:4}$ and 262 $F_{2:5}$ families revealed two recessive genes in Fallon, both of which are required to confer resistance to *D. pisi* isolate '22-P2033', and only one is required to confer resistance to *D. pisi* isolate '10728'. The gene required for resistance against both isolates is mapped to pea chromosome V based on molecular mapping using microsatellite markers. The other one is located on chromosome I.

Tightly linked markers for selecting the resistance genes have not been identified. A whole-genome sequencing approach to capture higher-density polymorphism between the parental lines and the resistant and susceptible bulks revealed three promising SNPs: Ch7997, C7SNP1, and C5SNP7, across three different pea genes. Further validation and molecular mapping of the SNPs are underway.

REFERENCES CITED

Ali, S., Nitschke, L., Dube, A., Krause, M., & Cameron, B. (1978). Selection of pea lines for resistance to pathotypes of Ascochyta pinodes, A. pisi and Phoma medicaginis var. Pinodella. Australian Journal of Agricultural Research, 29(4), 841–849. https://doi.org/10.1071/AR9780841

Annan, E. (2025). Exploration of the Genetic Resistance to Ascochyta Blight in Field Peas.

Annan, E. N., Nyamesorto, B., Yan, Q., McPhee, K., & Huang, L. (2023). Optimized High Throughput Ascochyta Blight Screening Protocols and Immunity to A. pisi in Pea. Pathogens, 12(3), 494. https://doi.org/10.3390/pathogens12030494

Bos, L., Hampton, R. O., & Makkouk, K. M. (1988). Viruses and virus diseases of pea, lentil, faba bean and chickpea. In R. J. Summerfield (Ed.), World crops: Cool season food legumes: A global perspective of the problems and prospects for crop improvement in pea, lentil, faba bean and chickpea (pp. 591–615). Springer Netherlands. https://doi.org/10.1007/978-94-009-2764-3_49

Bretag, T. W., Keane, P. J., & Price, T. V. (2006). The epidemiology and control of ascochyta blight in field peas: A review. Australian Journal of Agricultural Research, 57(8), 883–902. https://doi.org/10.1071/AR05222

Briar, S. S., Khan, M. R., & Zwart, R. (2023). Chapter 8—Nematode problems in pulse crops and their sustainable management. In M. Rahman Khan & M. Quintanilla (Eds.), Nematode Diseases of Crops and their Sustainable Management (pp. 183–204). Academic Press. https://doi.org/10.1016/B978-0-323-91226-6.00010-9

Current World Population: 8,298,978,817. (2026-04-21). World Population Review. https://worldpopulationreview.com/

Darby, P., Lewis, B. G., & Matthews, P. (1986). Diversity of virulence within Ascochyta pisi and resistance in the genus Pisum. Plant Pathology, 35(2), 214–223. https://doi.org/10.1111/j.1365-3059.1986.tb02006.x

Davidson, J. A., Wilmshurst, C. J., Scott, E. S., & Salam, M. U. (2013). Relationship between ascochyta blight on field pea (Pisum sativum) and spore release patterns of Didymella pinodes and other causal agents of ascochyta blight. Plant Pathology, 62(6), 1258–1270. https://doi.org/10.1111/ppa.12044

Domestication of Pulses in the Old World | Science. (n.d.). Retrieved April 16, 2026, from https://www.science.org/doi/10.1126/science.182.4115.887

Ellis, T. H. N., Poyser, S. J., Knox, M. R., Vershinin, A. V., & Ambrose, M. J. (1998). Polymorphism of insertion sites of Ty1-copia class retrotransposons and its use for

linkage and diversity analysis in pea. Molecular and General Genetics MGG, 260(1), 9–19. https://doi.org/10.1007/PL00008630

Ensembl Plants. (n.d.). Retrieved April 7, 2026, from https://plants.ensembl.org/index.html

Fondevilla, S., ÅVila, C. M., Cubero, J. I., & Rubiales, D. (2005). Response to Mycosphaerella pinodes in a germplasm collection of Pisum spp. Plant Breeding, 124(3), 313–315. https://doi.org/10.1111/j.1439-0523.2005.01104.x

Genetic Recombination and Gene Mapping | Learn Science at Scitable. (n.d.). Retrieved February 1, 2026, from http://www.nature.com/scitable/topicpage/thomas-hunt-morgan-genetic-recombination-and-gene-496

Home URGI - URGI. (n.d.). Retrieved March 27, 2026, from https://urgi.versailles.inrae.fr/

Jha, A. B., Gali, K. K., Tar'an, B., & Warkentin, T. D. (2017). Fine Mapping of QTLs for Ascochyta Blight Resistance in Pea Using Heterogeneous Inbred Families. Frontiers in Plant Science, 8. https://doi.org/10.3389/fpls.2017.00765

Jones, L. K. (1927). Studies of the Nature and Control of Blight, Leaf and Pod Spot, and Footrot of Peas Caused by Species of Ascochyta. New York State Agricultural Experiment Station.

Karkanis, A., Ntatsi, G., Kontopoulou, C.-K., Pristeri, A., Bilalis, D., & Savvas, D. (2016). Field Pea in European Cropping Systems: Adaptability, Biological Nitrogen Fixation and Cultivation Practices. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 44(2), 325–336. https://doi.org/10.15835/nbha44210618

Kiba, A., Takeda, T., Kanemitsu, T., Toyoda, K., Ichinose, Y., Yamada, T., & Shiraishi, T. (1999). Induction of Defense Responses by Synthetic Glycopeptides that Have a Partial Structure of the Elicitor in the Spore Germination Fluid of Mycosphaerella pinodes. Plant and Cell Physiology, 40(9), 978–985. https://doi.org/10.1093/oxfordjournals.pcp.a029631

Kraft, J. (1994). Fusarium wilt of peas (a review). Agronomie, 14(9), 561–567.

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., … Burstin, J. (2019a). A reference genome for pea provides insight into legume genome evolution. Nature Genetics, 51(9), 1411–1422. https://doi.org/10.1038/s41588-019-0480-1

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., … Burstin, J. (2019b). A reference

genome for pea provides insight into legume genome evolution. Nature Genetics, 51(9), 1411–1422. https://doi.org/10.1038/s41588-019-0480-1

Kropp, B. R., Matheny, P. B., & Nanagyulyan, S. G. (2010). Phylogenetic taxonomy of the Inocybe splendens group and evolution of supersection “Marginatae.” Mycologia, 102(3), 560–573. https://doi.org/10.3852/08-032

Lee, R. C., Grime, C. R., O’Driscoll, K., Khentry, Y., Farfan-Caceres, L. M., Tahghighi, H., & Kamphuis, L. G. (2023). Field Pea (Pisum sativum) Germplasm Screening for Seedling Ascochyta Blight Resistance and Genome-Wide Association Studies Reveal Loci Associated with Resistance to Peyronellaea pinodes and Ascochyta koolunga. Phytopathology®, 113(2), 265–276. https://doi.org/10.1094/PHYTO-02-22-0051-R

Loridon, K., McPhee, K., Morin, J., Dubreuil, P., Pilet-Nayel, M. L., Aubert, G., Rameau, C., Baranger, A., Coyne, C., Lejeune-Hènaut, I., & Burstin, J. (2005). Microsatellite marker polymorphism and mapping in pea (Pisum sativum L.). Theoretical and Applied Genetics, 111(6), 1022–1031. https://doi.org/10.1007/s00122-005-0014-3

Lupwayi, N. Z., & Kennedy, A. C. (2007). Grain Legumes in Northern Great Plains: Impacts on Selected Biological Soil Processes. Agronomy Journal, 99(6), 1700–1709. https://doi.org/10.2134/agronj2006.0313s

Lyall. (1958). THE INHERITANCE OF RESISTANCE TO ASCOCHYTA PISI LIB. IN PEAS. https://doi.org/10.4141/cjps58-035

McDonald, G. K., & Peck, D. (2009). Effects of crop rotation, residue retention and sowing time on the incidence and survival of ascochyta blight and its effect on grain yield of field peas (Pisum sativum L.). Field Crops Research, 111(1), 11–21. https://doi.org/10.1016/j.fcr.2008.10.001

Nimbalkar, V., Chikte, D., Raman, T., Maniyar, S., & Gaikwad, P. (n.d.). GENE MAPPING: BASICS, TECHNIQUES AND SIGNIFICANCE.

Owati, A., Agindotan, B., & Burrows, M. (2019). Development and Application of Real-Time and Conventional SSR-PCR Assays for Rapid and Sensitive Detection of Didymella pisi Associated with Ascochyta Blight of Dry Pea. Plant Disease, 103(11), 2751–2758. https://doi.org/10.1094/PDIS-02-19-0381-RE

Pandey, B. K., Singh, U. S., & Chaube, H. S. (1987). Mode of Infection of Ascochyta Blight of Chickpea Caused by Ascochyta rabiei. Journal of Phytopathology, 119(1), 88–93. https://doi.org/10.1111/j.1439-0434.1987.tb04387.x

Prioul, S., Frankewitz, A., Deniot, G., Morin, G., & Baranger, A. (2004). Mapping of quantitative trait loci for partial resistance to Mycosphaerella pinodes in pea (Pisum sativum L.), at

the seedling and adult plant stages. Theoretical and Applied Genetics, 108(7), 1322–1334. https://doi.org/10.1007/s00122-003-1543-2

PulseDB. (n.d.). Retrieved March 27, 2026, from https://www.pulsedb.org/

Ram, H., Hedau, N. K., Chaudhari, G. V., & Kant, L. (2021). Peas with zero shelling edible pods: A review. Scientia Horticulturae, 288, 110333. https://doi.org/10.1016/j.scienta.2021.110333

Semagn, K., Bjørnstad, Å., & Ndjiondjop, M. (2007). Principles, requirements and prospects of genetic mapping in plants. African Journal of Biotechnology, 525, 2569–2587.

Shao, D., Smith, D. L., Kabbage, M., & Roth, M. G. (2021). Effectors of Plant Necrotrophic Fungi. Frontiers in Plant Science, 12, 687713. https://doi.org/10.3389/fpls.2021.687713

Sivachandra Kumar, N. T., & Banniza, S. (2017). Assessment of the Effect of Seed Infection with Ascochyta pisi on Pea in Western Canada. Frontiers in Plant Science, 8, 933. https://doi.org/10.3389/fpls.2017.00933

Sivachandrakumar, N. T. (2016, December 1). Evaluation of the relative importance of Ascochyta pisi in the Ascochyta blight complex of pea in Saskatchewan. https://www.semanticscholar.org/paper/Evaluation-of-the-relative-importance-of-Ascochyta-Sivachandrakumar/410221b83de82ef6ef58aae9e0311bf797040d13

Skoglund, L. G., Harveson, R. M., Chen, W., Dugan, F., Schwartz, H. F., Markell, S. G., Porter, L., Burrows, M. L., & Goswami, R. (2011). Ascochyta Blight of Peas. Plant Health Progress, 12(1), 29. https://doi.org/10.1094/PHP-2011-0330-01-RS

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

Smýkal, P., Coyne, C. J., Ambrose, M. J., Maxted, N., Schaefer, H., Blair, M. W., Berger, J., Greene, S. L., Nelson, M. N., Besharat, N., Vymyslický, T., Toker, C., Saxena, R. K., Roorkiwal, M., Pandey, M. K., Hu, J., Li, Y. H., Wang, L. X., Guo, Y., … Varshney, R. K. (2015). Legume Crops Phylogeny and Genetic Diversity for Science and Breeding. Critical Reviews in Plant Sciences, 34(1–3), 43–104. https://doi.org/10.1080/07352689.2014.897904

Taylor, J. D., Teverson, D. M., Allen, D. J., & Pastor-Corrales, M. A. (1996). Identification and origin of races of Pseudomonas syringae pv. Phaseolicola from Africa and other bean growing areas. Plant Pathology, 45(3), 469–478. https://doi.org/10.1046/j.1365-3059.1996.d01-147.x

Timmerman-Vaughan, G. M., Frew, T. J., Russell, A. C., Khan, T., Butler, R., Gilpin, M., Murray, S., & Falloon, K. (2002). QTL Mapping of Partial Resistance to Field Epidemics of Ascochyta Blight of Pea. Crop Science, 42(6), 2100–2111. https://doi.org/10.2135/cropsci2002.2100

Tran, H. S., You, M. P., Khan, T. N., & Barbetti, M. J. (2015). Relative Host Resistance to Black Spot Disease in Field Pea (Pisum sativum) is Determined by Individual Pathogens. Plant Disease, 99(5), 580–587. https://doi.org/10.1094/PDIS-06-14-0655-RE

Wallen, V. R. (1957). The identification and distribution of physiologic races of ascochyta pisi lib. In canada. Canadian Journal of Plant Science, 37(4), 337–341. https://doi.org/10.4141/cjps57-039

Wang, H., Hwang, S. F., Chang, K. F., Turnbull, G. D., & Howard, R. J. (2000). Characterization of Ascochyta isolates and susceptibility of pea cultivars to the ascochyta disease complex in Alberta. Plant Pathology, 49(5), 540–545. https://doi.org/10.1046/j.1365-3059.2000.00489.x

Wang, X., Jiang, N., Liu, J., Liu, W., & Wang, G.-L. (2014). The role of effectors and host immunity in plant–necrotrophic fungal interactions. Virulence, 5(7), 722–732. https://doi.org/10.4161/viru.29798

Wark, D. C. (1950). The inheritance of resistance to Ascochyta pisi Lib. In Pisum sativum L. Australian Journal of Agricultural Research, 1(4), 382–390. https://doi.org/10.1071/AR9500382

Wu, D.-T., Li, W.-X., Wan, J.-J., Hu, Y.-C., Gan, R.-Y., & Zou, L. (2023). A Comprehensive Review of Pea (Pisum sativum L.): Chemical Composition, Processing, Health Benefits, and Food Applications. Foods, 12(13), 2527. https://doi.org/10.3390/foods12132527

Zhang, R., Hwang, S.-F., Chang, K.-F., Gossen, B. D., Strelkov, S. E., Turnbull, G. D., & Blade, S. F. (2006). Genetic Resistance to Mycosphaerella pinodes in 558 Field Pea Accessions. Crop Science, 46(6), 2409–2414. https://doi.org/10.2135/cropsci2006.02.0089

APPENDIX: PRIMER SEQUENCES FOR SELECTED VARIANTS

Table A1. Primer sequences for variant sites

Name	Sequences	Tm (°C)	Chromosome
3LGSNP1F	GCCAGAATGGATCGTACGTTAGA	62	V
3LGSNP1R	CGGCAACGCCAATTATTC		
3LGSNP2F	CTGATGCAAGCTCAGTT	59	V
3LGSNP2R	GGTTCTGTAGTTCTACACAC		
6LGSNP4F	CCAGTTAAAAGCTCAAACCG	58	I
6LGSNP4R	GGCCGTATTTATGCATG		
2LGOC1F	CCTCCATCACCATGTACCTGT	63	VI
2LGOC1R	GCGATTCTCACAGGTTTGCC		
3LGOC1F	GCAGCTAGCTGAACAACTGG	63	V
3LGOC1R	GCAAGTTCTTCGTTAAGCCGT		
Ch6452F	GAGGAAGAAGCAAGGCACGA	64	VI
Ch6452R	AGATGCCTAAGACTCCCGGT		
Ch1452F	GTGACACCTTCCAGATGCCA	64	I
Ch1452R	TCACCAGTTGGCATGCATGA		
Ch2452F	TGCCGATCCAAATCTGCCAT	64	II
Ch2452R	GTCCTCCACCAGTTGACACG		
Ch2452.1F	GAGGAAGAAGCAAGGCACGA	64	II
Ch2452.1R	GAGGTTGTCGATCCGCTCAT		
Ch7997F	GGG GAA AAA ATA CGT ACT GCT	63.5	VII
Ch7997R	GAAACATGGGGGATGAGAAGGTG		

Ch7763F Ch7763R	AGGTCCAAATAACCGGGCTC GGTGTTGGAAGGGTTGTGAGCT	63	VII
Ch1756F Ch1756R	CAACGGAGAGGTGACCAGAC CTGGTTGAGTCTTCCCGGTC	63.8	I
C6SNP1F C6SNP1R	CAAAGCACCAAAAAGGGCCA CAGGAAACACAACTGCTGCC	64	VI
C7SNP1F C7SNP1R	ACCTTGTCCACCAGTGAACA GGTTCCTGTTGATTCTGCTGG	63.3	VII
C7SNP2F C7SNP2R	CATGCCCTGGATCAAGAGGA TCGTGAAGGGAGGTCTTTTGG	63.5	VII
C7SNP3F C7SNP3R	ACTTGCAGGTAAGCATGGCT CAACCATGCATCATCCAGCA	63.2	VII
C7SNP4-5F C7SNP4-5R	GCATAGGCAATGGAATCTCTGT CCGCCGACACATGTCAGATA	63	VII
C7SNP6F C7SNP6R	AGGGGGAGAGAATTGTAGGAGT AAGCTCTCCTTAAGTCATATTC	59	VII
C7SNP7F C7SNP7R	CATGAGGAGGTGAAAAGA CTTTCTTCATCCCGAGATCAC	58	VII
C5SNP1F C5SNP1R	GATTTGGGATTTGGGGTTTTG AGCCAATCCGATACTGAGCAC	62	V

C5SNP2F C5SNP2R	CTCTAAAGCGCGTGCAGTTC CCGTGAACACAAGTCAAGCC	63.4	V
C5SNP3-4F C5SNP3-4R	AGTAAGTGTGCCGCTAATTGG AAGGAGCGAAGGGTAGAGGA	63	V
C5SNP5-6F C5SNP5-6R	TCCTTTGTGGAGGGTTAGAGT TCAGGGTTACACACAGTGGT	63	V
C5SNP7F C5SNP7R	GGGATCGTCACGAACCTGTT ACTGGGATCCCTCCGTAGTT	64	V
C5SNP8F C5SNP8R	AGATCGTCAATTTTCAACTGGA ATCCCTCTTCCCGTTTCGTC	63	V
C5SNP9F C5SNP9R	CCGGCGTATACTGGAATGGT TATCGAGCTCAACCAACCCC	63.6	V
C5SNP10F C5SNP10R	GCGCAGGAACAGATTTCTGTC CACAAAACACACTTTCTGGC	62	V