INHERITANCE OF PARTIAL RESISTANCE TO WHITE MOLD IN FIELD PEA (*Pisum sativum* L.)

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MASTER OF SCIENCE

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ABSTRACT

Sclerotinia sclerotiorum causes white mold and severe yield losses of pea. Four hundred eighty-four accessions from the *Pisum* core collection were screened for resistance using a mini-agar plug technique. Forty-nine, 41, and 13 accessions were identified with partial resistance based on lesion expansion inhibition (LEI), nodal transmission inhibition (NTI), and both traits combined, respectively. A genetic linkage map based on F_2 DNA from the cross, Lifter/PI240515, was developed with 78 markers on nine linkage groups (LG) spanning 734 cM. Two quantitative trait loci (QTL) were identified based on phenotypic data from $F_{2:3}$ and $F_{3:4}$ families. A single QTL on LGIII explained 34.1% of the phenotypic variation for LEI, while a second QTL on LGII(b) explained 2.5% of the phenotypic variation for NTI. This is the first report of QTL for *S. sclerotiorum* resistance in pea which will be useful in development of resistant pea varieties.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Field Pea Production in the Northern Plains

Cereals and legumes contribute the majority of the calories and essential nutrients to human and animal diets (Isely, 1982). Compared to grasses, legumes produce seeds that are richer in protein, essential vitamins, minerals, and dietary fiber. Far more legume genera and species are cultivated than cereals around the world (Isely, 1982). In addition to their nutritional benefits they are grown for production of resins, gums, ornamentals, lumber, fiber, and flavorings. Specific incentives of integrating legumes to agricultural systems are soil enrichment through biological nitrogen fixation and disease control when used in rotation.

The Northern Plains region produces several important legume crops such as soybean (*Glycine max* L.), dry bean (*Phaseolus vulgaris* L.), alfalfa (*Medicago sativa* L.), lentil (*Lens culinaris* Medik.), chickpea (*Cicer arietinum* L.) and pea (*Pisum sativum* L.). The latter three species are known as pulse crops. The name 'pulse' comes from Latin word 'puls' meaning thick soup prepared using soaked grains of these crops. The Food and Agricultural Organization (FAO) categorizes pulse crops as grain legumes grown for human and livestock consumption.

The past decade has seen the Northern Plains region emerge as a leader in pulse crop production. Among pulse crops dry pea commands the greatest area planted and harvested in the Northern Plains. North Dakota has led the region and nation in field pea production since 2001 based on area and volume (Table 1.1). Historically, the crop was primarily grown in the Palouse region of Washington and Idaho. According to the USDA National Agricultural Statistics Service (NASS), field peas were planted on 34400 hectares in ND in 2011.

Table 1.1 Field pea production estimates for major states as of November 1, 2010.

	Area harvested									
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
State	ha									
ID	9,307	16,187	21,853	22,257	18,615	11,735	9,712	14,568	16,592	12,140
MT	8,498	10,926	12,545	25,495	49,371	77,294	87,816	93,482	91,458	83,769
ND	34,802	58,461	62,726	119,786	208,413	238,764	202,342	202,342	194,249	161,874
OR	1,942	1,821	2,630	2,751	1,982	3,277	1,740	2,144	2,387	2,589
WA	25,090	30,756	33,184	35,207	31,565	26,709	26,709	30,351	34,398	27,518
U.S.	79,642	11,537	132,939	205,499	309,948	357,782	328,321	342,890	339,086	287,893
	Yield									
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
State	kg ha $^{-1}$									
ID	2,240	2,016	1,344	1,904	1,456	1,792	1,904	1,680	2,128	1,792
MT	1,568	896	1,624	2,251	2,016	1,210	1,904	1,210	1,490	2,240
ND	2,262	1,792	1,982	2,620	2,128	1,770	2,330	1,770	2,688	2,274
OR	1,120	1,568	2,240	3,360	2,240	2,296	2,576	2,856	2,509	3,304
WA	2,240	2,240	1,680	2,688	1,904	2,016	2,218	1,792	2,240	2,128
U.S.	2,150	1,855	1,774	2,519	2,047	1,672	2,195	1,622	2,290	2,239

Source: Crop Production Summary, USDA-NASS

Pea is grown for harvest of immature seed or dried seed. In the Northern Plains, pea is produced for dried seeds and can be divided into 4 market classes: green cotyledon, yellow cotyledon, Austrian winter and marrowfat. The dry seed of pea is mainly produced for feed. Green and yellow cotyledon peas are primarily produced for livestock, but are also marketed as split peas for human consumption. Marrowfat pea is grown for confectionary, and Austrian winter pea is grown for specialty markets such as birdseed, forage and green manure.

All cultivars of field pea grown for different market classes belong to *Pisum sativum* L. Taxonomy of *P. sativum* divides the species into five subspecies and five varieties (Xu-xiao et al., 2009). The only other species in the genus is *Pisum fulvum* (Table 1.2). All cultivars in the genus are annual climbing or semi-bushy, herbaceous plants that exhibit a wide spectrum of form and habit (Davies et al., 1981). The cultivated *Pisum* species such as *P. sativum* var. *sativum,* known as field pea, is mostly grown for seed while *Pisum sativum* is grown for green pods and

consumed as vegetables (Duke, 1981; Davies et al., 1985). Both varieties are grown in the temperate areas of the world.

Pea was domesticated along with other pulse crops in association with cereal crops such as wheat (*Triticum aestivum* L.) and barley (*Hordum vulgare* L.) in the Fertile Crescent 7000 to 8000 years ago (Zohary and Hopf, 1973). Domestication of pea, as in other crops, resulted in a genetic 'bottleneck', therefore wild relatives of pea represent potential genetic diversity that can be exploited in cultivated types to overcome biotic and abiotic stress (Harlan, 1976; Muehlbauer and Kaiser, 1994). Wild pea still exists in nature in this region. Comparison of wild relatives found in nature to cultivated pea show clear signs of domestication; i.e. alterations in plant habit, pod dehiscence, seed size, seed dormancy and flowering habit (Weeden, 2007). In addition, cultivated pea has shorter internodes, upright posture, semi-leafless morphology and larger root systems.

Table 1.2 Taxonomy of *Pisum* genus based on Xu-xiao et al., (2009).

Species	Subspecies	Variety	Common name	Gene pool
Pisum sativum L.	ssp. *sativum*	var. *sativum*	Garden pea	Primary
		var. *arvense* (L.) Poir	Dry pea	Primary
	ssp. *elatius*	var. *elatius* (M. Bieb) Alef		Primary
		var. *pumilio* Mzikle		Primary
		var. *humile* Boiss and Noe		Primary
	ssp. *abyssinicum*			Primary
	ssp. *asiaticum*			Primary
	ssp. *transcaucasicum*			Primary
Pisum fulvum Sibth and Sm.			Wild pea	Secondary

Both species of Pisum are diploid with 2n=2x=14 chromosome number. *P. sativum* subspecies can be crossed easily and generally yield 5-7 viable seeds which are relatively high

among pulse crops. Hybrids between *P. sativum* and *P. fulvum* are achievable when the latter is used as a pollen source (Muehlbauer, 1991). Conventional pea breeding programs have utilized *P. sativum* and *P. fulvum* gene pools extensively in search of desired traits.

Pea can be grown in a broad range of soil types, from light sandy to heavy clay as long as standing water and high salinity are avoided. Cultivated pea has an annual moisture requirement of 500-800 mm. Well drained soils are preferred for pea production (Duke, 1981). Exposure to water logged conditions will reduce crop vigor and often result in plant death. Irrigation is not needed in temperate areas of pea production. However, irrigation at the start of flowering and during pod filling has shown significant increase in yield (Salter and Williams, 1967).

Field pea can be grown in no-till or conventional-till cropping systems. A firm, moist seed bed is essential for proper seed germination and stand establishment. Excessive tillage in the spring can result in drying out of the seedbed and poor germination. Pea germination requires more soil moisture than cereal grains. Field peas are typically seeded in rows spaced 15-30 cm apart using an air seeder or conventional grain drill. Seeding at 200-338 kg ha^{-1} depending on seed size at the depth of 2-8 cm is recommended for field pea (Duke, 1981). Soil bed should be smooth enough to plant seeds at a consistent depth.

Field pea, like other cool season legumes, should be sown early in the growing season. Planting in April to mid-May is optimal and will result in flowering during cooler weather in June and early July. Seeding peas beyond mid-May delays flowering and increases the risk of heat stress and disease, such as powdery mildew, resulting in reduced yields (Duke, 1981). Most pea varieties require at least 85 days post-emergence to reach full maturity. The indeterminate types can take as much as 110 days or more to reach maturity. In North Dakota, field pea takes

about 60 days from seeding until flowering and 90 to 100 days to reach maturity under typical weather conditions (Akyuz et al., 2010; Schatz and Endres, 2009).

Field pea will fix the majority of required nitrogen in the presence of *Rhizobium leguminosarium,* thus no nitrogen fertilization is needed. If the pea crop is grown in a field for the first time, inoculum should be applied with the seed. Field peas are most often rotated with small grain crops. Residual nitrogen will also be present for the succeeding crop and will reduce fertilization costs. It is estimated that the pea crop retains approximately 50 kg ha^{-1} of nitrogen to the soil (Davies et al., 1985).

Pea production in the Northern Plains is affected by many pathogens. The yield and seed quality of field pea is affected by fungal diseases such as ascochyta blight *(Ascochyta pisi, Ascochyta pinodes),,* powdery mildew *(Erysiphe pisi),* fusarium wilt (*Fusarium oxysporum),* downy mildew *(Peronospora pisi),* pre-emergence dampingoff *(Phythium* spp.), grey mold *(Botrytis cinerea*), common root rot *(Fusarium* spp.*),* black root rot *(Thielaviopsis basicola),* and white mold (*Sclerotinia sclerotiorum)*. Viruses that attack pea include: pea early browning virus (PEBV), pea enation mosaic virus (PEMV), pea mosaic virus (PMV), pea top yellows (PTY), pea seed-borne mosaic virus (PSbMV), and pea streak virus (PSV). Economically, the most important bacterial disease of pea is bacterial blight caused by *Pseudomonas pisi* (Duke, 1981; Davies et al., 1985).

Insect pests include pea aphid *(Acyrthosiphon pisum),* pea thrips *(Kakothrips robustus)*, pea seed beetle *(Apion* sp.), pea weevil *(Bruchis pisorum),* bean weevil (*Sitona lineatus)*, pea midge (*Contarina pisi)*, pea pod borer *(Diachrysia oblique)*, pea moth (*Cydia nigricana)*, leaf minor (*Phytomuza horticola)*, American bollworm *(Heliothis Zea),* bean fly (*Ophiomyia phaseoli)*, *spider mites* (*Tetranychus* sp.). Several parasitic nematode

species such as root lesion nematode *(Pratylenchus penetrants)*, stem nematode (*Ditylenchus dipsaci)*, pea cyst nematode (*Heterodera goettingiana)*, and root knot nematode (*Meloidogyne javanica),* adversely affect pea production (Duke, 1981; Davies et al, 1985).

Pea seeds are rich in protein, vitamins, minerals, and fiber. On average, dry pea contains: 10.9% water, 22.9% protein, 1.4 % fat, 60.7% carbohydrate, 1.4% crude fiber and 2.7% ash (Duke, 1981). The protein concentration in seeds may vary from 15.5-39.7% (Davies et al., 1985). Relatively high protein content in pea compared to seeds of other crops makes pea a good protein source for many people around the world. In order to get the most nutritional benefit from pea, seeds should be soaked overnight and cooked for 1-2 hours (Muehlbauer et al., 1983). Prolonged soaking reduces anti-nutritional factors found in the pea grain and enhances the texture for palatability. As a nutritious legume that is rich in protein, fiber, minerals, and vitamins, pea is desired for its nutritional properties putting it in positive position as a health food. Increased concerns about personal health, food quality and safety, as well as interest in vegetarianism and exotic dishes in the general public is expected to trigger increase in pulse consumption (Schneider, 2002).

Research on pea conducted in North Dakota targets identification and improvement of key agronomic components of pea and introducing new desirable traits. These traits are aimed to make pea better suited to withstand the abiotic and biotic stresses present in the Northern Plains region. Identification of genetic resources to improve seed nutritional content and baking quality is also a priority.

In one study, genotypes with very high selenium content were discovered; an attribute that can reduce malnutrition in developing countries (Thavarajah, personal communication,

2011). In addition, pioneering research is being conducted to develop winter hardy pea varieties for the Northern Plains region (McPhee, personal communication, 2011).

Previous trials in Pacific Northwest region demonstrated that fall-seeded winter pea produced as much as 1830 kg ha^{-1} more seed yield than spring sown pea (Chen et al., 2006). Currently suitable seeding date for the state is being investigated. It is thought that a longer growing season enabling winter peas to develop more branches and develop faster in the early spring will result in increased yield.

Sclerotinia sclerotiorum, the Causal Agent of White Mold in Pea

Sclerotinia sclerotiorum (Lib.) de Bary, the causal agent of white mold, is found in many regions of the world. The pathogen is a homothallic fungal species and it is classified as a member of Amastigomycota, class Discomycetes, order Helotiales, and family Sclerotiniaceae (Purdy, 1979). The scientific name - *Sclerotinia sclerotiorum* (Lib.) de Bary, has been changed several times since the species was identified by Whetzel (1945) and genera in the family Sclerotiniaceae went through redistribution (Korf and Durmont, 1972).

Two other identified species of *Sclerotinia, Sclerotinia trifoliorum* and *Sclerotinia minor,* differ in morphology and pathogenicity. *S. trifoliorum* is known to infect only some forage legumes in the southeast and eastern United States, while *S. minor* primarily infects lettuce and peanut. Morphologically, *S. trifoliorum* is more similar to *S. sclerotiorum* than *S. minor* (Kohn, 1979). The latter produces much smaller sclerotia and generally produces apothecia less frequently in nature.

Sclerotinia sclerotiorum is capable of infecting over 400 plant species worldwide (Bolton et al., 2006). In North Dakota, the pathogen infects pulse crops as well as other major crops

grown in the state such as soybean [*Glycine max* (L.) Merr.], sunflower (*Helianthus annuus* L.), common bean (*Phaseolus vulgaris* L.), and canola (*Brassica campestris* L.) thus, crop rotation involving these crops and pea is relatively ineffective in controlling the disease.

Other crops grown in the Northern Plains which can also become opportunistic hosts to *S. sclerotiorum* include flax (*Linum usitatissimum* L.), potato (*Solanum tuberosum* L.), sugarbeet (*Beta vulgaris* L.), safflower (*Carthamus tinctorius* L.), and alfalfa (*Medicago sativa* L.). Many weeds found in the region such as marsh elder (*Iva frutescens* L.), pigweed (*Amaranthus hybridus* L.), Canada thistle (*Cirsium arvense* L.), sow thistle (*Sonchus arvensis* L.), and wild mustard (*Synapsis arvensis* L.) are also hosts and can play a role in disease cycles (Bolton et al., 2006).

Among pulse crops pea is highly susceptible to *S. sclerotiorum*. The fungus is responsible for white mold disease and is also known by other names such as cottony rot, watery soft rot, white rot, and crown rot (Bolton et al., 2006). The pea plant can be infected through any plant organ and the pathogen can spread through soil or air. Generally, infection can be initiated at any stage during the growing season if moisture and temperature requirements are met and causes mid-stem, leaf, and pod rot. In the soil, germinated sclerotia can initiate mycelial infection of pea roots and stems and cause stem rot. *S. sclerotiorum* causes epidemics during later stages of the pea growing season when plants are flowering and the canopy is closed.

Observable symptoms of infected plants include soaked lesions with cottony mycelial growth on infected stems, leaves or pods. Advanced disease causes wilting, shredded branches and dead plants with decay. Diseased plants that survive produce shriveled seeds and sclerotia that can completely replace seeds and internal parts of the plant stem. Destructive physiological effects of the pathogen include reduced photosynthetic surface area, disrupted water and nutrient

transport, and respiration aberrations due to modified stomata cells. These effects combine to reduce the yield potential of the pea crop. In susceptible crops, such as bean and pea, *Sclerotinia* infestation under optimal conditions can cause yield reductions as high as to 100% (Purdy, 1979).

Presently, all major broadleaf cultivars including pea grown in the Northern Plains are susceptible to *S. sclerotiorum*. An integrated approach to manage white mold in all host crops is recommended and necessary. Crop rotation involving non hosts, inoculum free seed, disease escape mechanisms and fungicide applications should be incorporated. White mold infestations and epidemics in all major host crops are sporadic and disease broadcasting has limited use. High humidity and cooler temperatures are major indicators of favorable conditions for disease infestations.

Pathogenesis of *S. sclerotiorum*

Life Cycle and Epidemiology

The life cycle of *Sclerotinia sclerotiorum* is relatively simple compared to many fungal pathogens. In temperate areas of the world such as the Northern Plains, *S. sclerotiorum* overwinters as sclerotia, solid dormant specks that can survive for up to seven years in soil and spread in the field via any soil movement such as tillage (Adams and Ayers, 1979; Bolton et al., 2006). Sclerotium is the inoculum source in the field and plays a central role in the epidemiology of white mold in host crops. The disease cycle begins when sclerotial dormancy is broken. Sclerotia are covered with a rind - black protective coat that contains melanin pigments. The rind makes the structure highly resistant to abiotic degradation and microbial attack. Medulla, the inner part of sclerotia, is a yellow-white aggregate of fungal cells rich in β-glucans and proteins

(Nelson, 1998). These cells will give rise to formation of myceliogenic hyphae or apothecial stalks during germination. Depending on environmental conditions such as soil temperature, moisture, and availability of a food source preconditioned sclerotia will germinate carpogenically or myceliogenically.

Hyphae are produced during myceliogenic germination of sclerotia and can directly attack any plant organs that the pathogen comes in contact with. These hyphae can grow and infect plant organs such as stem and leaves that come in contact with soil in lodged canopies. Mycelium of *S. sclerotiorum* can penetrate the cuticle of the host plant using enzymes and mechanical force via an infection peg or appressoria (Lumsden, 1976). Once inside the host, infectious hyphae advance through intercellular spaces of parenchyma tissue by disintegrating the middle lamella of host cells or through xylem tissue. Host cell death is followed by consumption of nutrients released from degraded host cells and further advancement of hyphae through intracellular spaces (Lumsden, 1979).

It has been reported that oxalic acid produced by *S. sclerotiorum* during infection is involved in deregulating guard cell function causing irregular stomata opening in advance of invading hyphae. Thus, host penetration can also occur through the stomata (Lumsden, 1979). In addition to causing water stress, open stomata were used by *S. sclerotiorum* for hyphal emergence and secondary colonization of the host plant as well as formation of sclerotia on the host surface.

Apothecia are produced during carpogenic germination of sclerotia that subsequently produce ascospores that infect above ground host plant organs. Carpogenic germination from sclerotia takes place when apothecia are produced. The environmental conditions that trigger carpogenic germination are variable. Soil temperature between 15-18 °C and high moisture level

are the most favorable for carpogenic germination. Once the germination is initiated a funnel-shaped apothecia, approximately 1 to 3 cm in size, is formed on sclerotia that are close to the soil surface.

Carpogenic germination of the fungus begins with formation of apothecium that produces ascospores and completes the sexual cycle. The fungus does not produce conidia though production of spermatia and microconidia with vague biological function has been reported. Ascus mother cells form hymenium that go through meiosis to form haploid germ cells. Clamp connections are formed between the apical cell and basal cell of the resulting ascogenous hyphae, allowing both cells to become binucleate. The apical cell develops into a young ascus, the nuclei fuse, meiosis takes place, followed by two rounds of mitotic division, which result in eight binucleate ascospores. The haploid chromosome number for *S. sclerotiorum* is eight. Mature apothecia release ascospores to the air in a process called 'puffing'. Traveling through the air enables the fungal spores to efficiently disperse the pathogen to a wide area (Steadman and Nickerson, 1975). Germinated sclerotia can produce ascospores for up to ten days in the field (Steadman, 1979). Spores that land on a plant surface remain viable for about two weeks. Spores become unviable quickly when temperature increases and relative humidity decreases. Due to microclimate effects, spores deposited on lower leaves of peas have better survival rate than spores deposited on the topmost leaves.

Ascospores are covered by sticky mucilage which aids in adhesion to the substrate they land on. Ascospore survival is reduced when exposed to humidity fluctuations and ultraviolet light (Clarkson et al., 2003). Spores germinate by producing germ tubes when plant tissue surfaces free of moisture reach temperatures of 20-25 °C (Abawi and Grogan, 1979).

Ascospores can germinate on the surface of healthy tissue, but cannot infect the plant without an exogenous nutrient source and adequate moisture. The germ tube must colonize an exogenous nutrient source first before infection can take place. Therefore, senescent or necrotic tissues generally serve as the initial nutrient source to initiate ascospore germination (Abawi and Grogan, 1979). In some legume crops, such as bean and pea, senescent petals during the flowering stage are primary nutrient sources for the ascospores, as are senescent or wounded tissues.

After the ascospore germinates, mechanical penetration through the cuticle of the plant happens through formation of a dome-shaped cushion from which an appressorium arises. Once inside the plant, fungal hyphae developed from the ascospore germ tube will follow the same pattern of hyphae attack in myceliogenic germination; first quickly spreading through intercellular spaces followed by branching hyphae that advance into intracellular spaces of invaded host tissue (Lumsden, 1979).

Oxalic Acid and Pathogenesis Enzymes

Necrotrophic pathogenesis and the life cycle of *Sclerotinia sclerotiorum* is facilitated by secretion of various pathogenesis chemicals by the organism such as oxalic acid, enzymes, and pectins (Marciano et al., 1983; Riou et al., 1991). Pathogenesis chemicals allow the pathogen to kill plant cells, penetrate plant tissues, and colonize host plants during the pathogen-host interaction. Combination of these compounds enables degradation of pea plant cell walls, providing nutrients for the fungus.

Among these chemicals oxalic acid is the main pathogenesis element as it creates a framework for other metabolites such as cell wall degrading enzymes (Godoy et al., 1990;

Lumsden 1979). Mutant strains of *S. sclerotiorum* that cannot produce oxalate are nonpathogenic on all host plants and are also unable to develop sclerotia (Godoy et al., 1990).

It is suspected that oxalate secretion enhances *S. sclerotiorum* virulence in several ways (Dutton and Evans, 1996; Bolton et al., 2006). Secretion of oxalic acid by *S. sclerotiorum* into the invaded host tissue provides with low pH that is required for optimal cell wall degradation by enzymes such as polygalacturonase (Bateman and Beer, 1965; Rollins and Dickman, 2001). In addition, pH change by oxalic acid activates sclerotial formation through several metabolic pathways (Rollins, 2001).

Oxalic acid can also degrade or weaken the plant cell walls via acidity or chelation of cell wall cations such as Ca^{2+} (Bateman and Beer, 1965). Accumulation of oxalic acid in plant vessels such as xylem disrupts water transportation at the infection points and results in host wilt. Along with oxalic acid, the pathogen releases many types of cell wall degrading enzymes upon infection. Three key pectolytic enzymes that are produced by *S. sclerotiorum* are exo-polygalactorunase, endo-polygalacturonase, and pectin methylesterase (Lumsden, 1976). Endo-polygalacturonase is the first enzyme produced upon infection, and catalyzes partial hydrolysis of pectic material found in the middle lamellae between plant cells. Subsequently, exo-polygalactorunase is produced during the infection process and it cleaves pectin to galacturonic acid. Pectin methylesterase is related with lesion expansion and its activity is essential for the other two enzymes to function at high efficiency (Lumsden, 1976).

Disease Management of White Mold in Pea

S. sclerotiorum is an opportunistic, necrotrophic parasite that can effectively attack, kill and consume the pea plant. Pea and many other crops cultivated in the Northern Plains are highly

susceptible to white mold. In order to achieve maximum disease control of white mold on pea an integrated disease management approach is necessary. This method includes cultural practices, chemical control, and genetic resistance.

Cultural practices to control white mold of pea include sowing disease-free seed, using three to five year rotations with non-host crops, tillage, promoting an upright, open canopy and host weed management (Kraft et al., 1996; Kraft and Pfleger, 2001).
Sowing disease free pea seeds is the simplest and most effective way to avoid introduction of many diseases including white mold. In the Northern Plains region, pea seed for planting comes mostly from certified seed stocks free of sclerotia. However, in many developing countries it is common for growers to sow seeds from the previous harvest.

Several chemicals can be applied to seeds prior sowing to eliminate sclerotial introduction. Seed treatments include molybdenum and boron in association with Benomyl give significant control of white mold in pea (Kuleshova, 1990). In one study, seed treatment with carbendazim at 2.5 g kg^{-1} showed effective control of white mold in pea (Sugha, 1999).
Tillage operations have mixed results on reducing white mold infection. Tillage can increase unwanted sclerotial dissemination in the field, but it can also bring them to the soil surface where microbial attack on sclerotia is high (Abawi and Grogan, 1975). Techniques such as no-till may reduce the spread of soil borne sclerotia and has been reported to be useful in inoculum control in soybean production (Gracia et al., 2002).

In order to control white mold of pea, the crop should not be planted following highly susceptible crops such as sunflower or canola. In case pea needs to be planted following susceptible crops, the areas that are known to harbor large amounts of sclerotia in the soil should

be avoided. In addition, many broadleaf weeds that can serve as volunteer crop for developing disease bridge must be managed.

Disease monitoring is crucial in avoiding epidemic levels of disease infestation. There is no established disease forecasting system developed for pea. However, many similarities between white mold incidence and epidemiology of pea and bean exist. Disease incidence of white mold in both crops can increase dramatically during canopy closure and flowering period if favorable environmental conditions are met. Apothecia production is especially severe during the flowering period of both crops since senesced petals serve as an initial food source for ascospore germination. Therefore, disease forecasting methods in beans which include analyzing weather conditions, growing season, and canopy structure could be practical in disease forecasting of pea (McDonald and Boland, 2004).

Chemical control includes application of foliar fungicides containing active ingredients such as carbendazim, mefenoxam, prothioconazole, and trifloxystrobin during early stages of disease symptoms or flowering stage (Sugha, 2001; McMullen and Markell, 2010). These fungicides are aimed at targeting different stages of *S. sclerotiorum*. Some are designed to kill ascospores or apothecia while others can degrade sclerotia. Complete coverage of the diseased material is critical.

The aforementioned cultural practices are limited in effectiveness or expensive. Increase in production costs creates a competitive disadvantage to growers. Moreover, the over usage of fungicides can trigger development of tolerance in the pathogen and creates environmental concerns. To increase the efficiency of the integrated approach, a pea variety partially resistant to white mold with open canopy plant architecture will be important in the long-term control of the disease.

Pea varieties grown in the Northern Plains are very susceptible to the disease. Most pea varieties will produce large, dense canopies that tend to hold moisture and moderate the high day time temperature; a microclimate conditions that favors S. *sclerotiorum* pathogenesis (Schwartz et al., 1978; Abawi and Hunter, 1979; Schwartz et al., 1987). At present, several pea varieties possess disease avoidance mechanisms such as upright plant architecture aided by semi-leafless pea morphology that keeps plants mutually supported and well aerated (Grünwald et al., 2004). These structures reduce the risk of microclimate formation that is required for white mold establishment and advance (Du Vale et al., 2001).

In addition, early flowering in the growing season may allow pea plants to impede ascospore dissemination. Abundance of inoculum combined with the environment is highly conducive to disease and avoidance alone will not be effective. In such cases, genetic resistance will be important. Physiological resistance based on active or passive inhibition of white mold of pea can benefit the pea plant in case escape mechanisms fail. Presence of modest physiological resistance can be assumed after close observation of disease reaction. Disease lesions produced by *S. sclerotiorum* show yellow discoloration of the tissue surrounding the lesion. In addition, the lesion does not progress at the same rate indicating the presence of physiological changes the cells go through during infection.

Novel genetic resistance sources in many susceptible host crops such as soybean, dry bean, and sunflower have been reported. Several accessions with partial physiological resistance in dry bean (Schwartz and Otto, 2006; Miklas and Grafton, 1992), soybean (Hartman et al., 2000; Vuong et al., 2004), and sunflower (Vuong et al., 2004) have been discovered. Accessions with partial resistance in pea were also found (Blanchette and Auld, 1978, Porter et al., 2008).

Porter et al., (2008) reported presence of partially resistant pea accessions in the *Pisum* core collection with two distinct resistance mechanisms (Porter et al., 2008). In his investigation, partially resistant pea accessions demonstrated lesion expansion inhibition (LEI), or nodal transmission inhibition (NTI) or both. Observation of distribution for disease reaction in pea accessions indicated quantitative inheritance of the trait.

The current and previous studies have shown that even the most resistant genotypes will become infected and killed if optimal conditions for pathogenesis exist for prolonged periods. Partial physiological resistance may be most efficient when combined with avoidance mechanisms and cultural control practices that create environmental conditions less favorable for pathogen infection and growth (Schwartz et al., 2006; Miklas et al., 1992).

Pisum Genetic Mapping: Past and Present

Initial genetic studies accomplished by Mendel and later by several other classical geneticists contributed to our understanding of trait segregation, chromosomal arrangements and gene location. These investigations involved simple trait analysis using two way crosses, mutant phenotype analysis and assigning linkage groups (cit.). The morphological traits included more than 160 mutant genotypes being placed on seven linkage groups (Blixt, 1972). Linkage groups developed this way resulted in discrepancy in the size and number of linkage groups that were only revealed later with molecular techniques.

Linkage groups and markers were aligned against the corresponding chromosomes following karyotyping on pea established seven chromosomes, 2n=2x=14 (Blixt, 1958). During the 1980s and later isozymes and restriction fragment polymorphism (RFLP) markers were used to establish a base map of pea. Initially isozyme markers (Weeden and Marx, 1984; 1987) and

later RFLPs were used to construct genetic maps and align them to the framework map established by Blixt.

Molecular markers enabled construction of more accurate pea linkage groups and precise location of major agronomic traits. Polymerase chain reaction (PCR) based markers further improved the density and ease of genetic mapping in pea. Initially random amplified polymorphic DNA (RAPD) and amplified length polymorphism (AFLP) markers were extensively utilized (Timmerman et al., 1993; Timmerman-Vaughn et al., 1996). Later identification of repeated sequences in species led to development of subsequent techniques. High density maps utilizing short sequence repeat (SSR) markers were established (Burstin et al., 2001; Loridon et al., 2005). During this time several additional genes were identified and placed on the pea genome.

Modern genetic investigations in pea are mainly carried out to understand the nature of more complex traits with direct applications to breeding. These traits are controlled by several genes with major and minor effects and cannot be efficiently analyzed with conventional techniques alone. Taxonomic classification and polymorphism content of *Pisum* has become more evident with molecular markers analysis (Burstin et al., 2001). Advanced techniques in sequencing and bioinformatics techniques will further reveal subtle features of the *Pisum* genome. These techniques will enable pinpointing locations of putative genes with high precision and establishing syntenic relationship with other plant species.

A trait of interest such as white mold resistance in pea is complex and requires extensive molecular and phenotypic analysis in order to identify number, location, and magnitude of genes associated with the trait. Breeding pea cultivars for such complex traits is enhanced through

application of mapping and QTL analysis results. Linked markers to conferring QTL can be used in selection of particular genotypes during breeding processes such as backcrossing.

CHAPTER 2. SCREENING THE *PISUM* CORE COLLECTION FOR RESISTANCE TO *SCLEROTINIA SCLEROTIORUM*

Introduction

The field pea (*Pisum sativum* L.) is a cool season legume crop cultivated in many temperate areas of the world. In the western hemisphere, the Northern Plains region is a major pea production area. The local agriculture, in the region, benefits from inclusion of pea in several ways. Small grain crops such as wheat and barley are grown extensively in the region and rotation with pea helps disease breaks of these crops. As a legume pea fixes free nitrogen and enriches the soil. As a result residual nitrogen in the soil can be used by monocot crops such as wheat, barley and corn and reduces the cost of fertilization.

Pea seeds have high content of protein, vitamins, minerals, and fiber. It is included in human and animal diets for its high protein concentration. Seed protein content of up to 39.7% have been reported (Davies et al., 1985). On average 100 gr of dried peas contain: 10.9% water, 22.9% protein, 1.4% fat, 60.7% carbohydrate, 1.4% crude fiber, and 2.7% ash (Duke, 1981; Hulse, 1994). Balanced composition of essential nutrients makes pea seed appropriate ingredient in many salads, soups and snacks around the world.

Pea production in the region is affected by many abiotic and biotic stresses. Major abiotic stresses in the region include high winds, extreme temperatures, drought, flood, hail storms and other natural phenomena. Pests of pea include numerous fungal, bacterial, viral, insect and nematode pathogens. Among many fungal pathogens that affect pea production, *S. sclerotiorum* can be particularly damaging. *S. sclerotiorum* causes white mold of pea and is responsible for yield loss and inferior seed quality.

In the Northern Plains region integrated pest management (IPM) of white mold of pea includes cultural practices that reduce the inoculum level in fields by crop rotation with non-host crops such as cereals, seed treatment and applications of foliar fungicides. Timely and collective application of these practices can have effective results in controlling the disease but each of the IPM components has limiting factors.

Applications of fungicides at the right time can control white mold; however, sporadic incidence of white mold outbreaks limit disease forecasting. Fungicides are expensive and may reduce the net profit realized by the grower. In addition, fungicide application always poses environmental and human health hazards to some extent. Crop rotation is limited because *S. sclerotiorum* affects many crops in the region and inoculum of the pathogen remains viable in the soil for several years. The most economical and environmentally friendly method of combating white mold of pea is development of genetically resistant pea varieties.

Exotic pea accessions represent a great repository of genetic variation found in pea. Incorporation of plant introductions with desired traits in breeding projects has been achieved in many crops including pea (Harlan, 1976; Muehlbauer et al., 1998). It is hypothesized that wild relatives of cultivated crops have been exposed for wide spectrum of biotic and abiotic stresses and therefore contain more genetic resistance and tolerance. In addition wild relatives may possess other desirable traits that could be used in improving agronomic or nutritional values of varieties.

Developing new pea varieties resistant to white mold involves discovery of pea accessions with significant resistance to *S. sclerotiorum* and transforming the resistance into adapted varieties. The primary gene pool of *Pisum* consists of *P. sativum* ssp. *sativum, P. sativum* ssp. *elatius, P. sativum* ssp. *abyssinicum, P. sativum* var. *arvense,* and *P. sativum* var.

pumilio. Secondary gene pool includes *P. fulvum*. Conventional crossing techniques results in viable hybrids; however, when *P. fulvum* is crossed with *P. sativum,* the former needs to be the pollen source in order to obtain fertile seed.

Pea germplasm improvement through increasing tolerance to biotic and abiotic stresses and agronomic traits encompasses introduction of exotic germplasm for acquiring traits of interest. Conventional breeders understand that success in crop improvement is dependent upon available genetic resources. Currently, more than 5400 accessions are available from USDA-ARS, WPRIS pea germplasm collection. In order to minimize germplasm redundancy and to increase screening efficiency the *Pisum* core collection was established to identify subset of pea germplasm that represents most genetic diversity based on 26 traits (Coyne et al., 2005).

Figure 2.1. Seed morphology variation in a typical pea accession obtained from USDA-ARS, WPRIS.

The *Pisum* core collection comes from more than 67 countries and contains accessions in the primary gene pool of *Pisum sativum*. The collection contains accessions from all *Pisum* subspecies and several from *P. fulvum*. Previous studies conducted to analyze morphological

traits such as biomass and root production showed great genetic diversity within the collection (Muehlbauer and McPhee, 2001; McPhee, 2005).

Many techniques have been adapted to identify resistance to white mold in other crops. Most of the techniques include mycelial inoculation of stem and leaves under controlled environments. Some of these methods include the oxalic acid assay, cotyledon inoculation, excised stem test, detached-leaf assay, cut stem inoculation, cut-petiole inoculation, straw test and agar plug inoculation. These methods use different evaluation techniques as well (Otto-Hansen et al., 2009).

Resistance to white mold in the pea core collection can be evaluated in greenhouse and field conditions. The advantage of using a tightly a controlled greenhouse environment is the capability to estimate slight changes in plant morphology during disease reaction that can be attributed to physiological resistance. Field trials for disease resistance with multiple locations can give additional data such as yield that can be analyzed in correlation with disease incidence. In addition, field trials can be used to evaluate disease escape traits such as canopy integrity post inoculation that can be utilized in gene pyramiding for white mold resistance in pea.

Pisum core collection screening experiment involved testing pea accessions in strictly regulated greenhouse environment. The main objective of the experiment was to identify novel pea germplasm with significant levels of partial resistance to white mold within the *Pisum* core collection that can be used in pea breeding.

Materials and Methods

Germplasm

The *Pisum* core collection representing 504 accessions was obtained from the USDA-ARS, Western Regional Plant Introduction Station (WPRIS) in Pullman, WA. The core collection represents the working pea collection and represents the genetic diversity in the whole collection (Coyne et al., 2005). The collection consists of one species three subspecies, and two varieties. Table 2.1 summarizes the content of the germplasm that was tested in Fargo.

Table 2.1. *P. sativum* accessions tested for white mold resistance in Fargo.

Taxonomy	Core Collection	Failed to Germinate	Tested
Pisum sativum	427	11	415
Pisum sativum spp. *sativum*	15	1	14
Pisum sativum spp. *elatius*	36	1	35
Pisum sativum spp. *abyssinicum*	14	0	14
Pisum sativum var. *arvense*	5	0	5
Pisum sativum var. *pumilio*	3	1	2
Total	500	14	485

The passport information for each accession in the *Pisum* core collection is provided in Appendix. Six accessions labeled as *P. sativum* ssp. *sativum*, and four accessions labeled *P. sativum* ssp. *arvense* have been tested for resistance to white mold for the first time. The total set of 500 genotypes was divided into 10 sets of 50 genotypes. Four two-week old seedlings from each genotype were used for screening. A randomized complete block design (RCBD) was used in the mist chamber.

Pathogen Culture

In 2003, *S. sclerotiorum* sclerotia of isolate Scl02 were collected from pea cultivar 'Snake' in Quincy, WA by Dr. Lyndon Porter and used in his subsequent germplasm screening. Sclerotia of the same isolate were obtained from Dr. Lyndon Porter and used to screen the *Pisum*

core collection in Fargo, ND. Sclerotia were stored at 4 °C until used. Sclerotial dormancy was broken by placing sclerotia into a 10% bleach solution for 20 minutes, followed by rinses with distilled water. The rinsed sclerotia were placed in 95% ethanol for one minute, removed and briefly flamed. Surface sterilized sclerotia were plated onto sterile potato dextrose agar (PDA), in a 15 mm x 100 mm Petri dish (VWR International, LLC). The PDA was made by dissolving 39 g of Difco™ Potato Dextrose Agar (Becton, Dickinson and Co., USA) powder in 1 L of purified water, mixing and boiling for 1 minute followed by autoclaving at 121 °C for 15 minutes.

Inoculum used to initiate disease in pea accessions was prepared using composite agar (CA) media. CA was prepared by mixing 18.5 g of Difco™ PDA and 8.75 g of Difco™ oatmeal agar in 0.5 L of H_2O and autoclaving the solution at 121 °C for 15 min. Then sterile media was cooled to a warm temperature and then 20 ml of CA was poured to 20 Petri dishes in a laminar flow fume hood and allowed to cool. Mycelia samples were taken from the initial cultures on PDA and subcultured on nine fresh Petri dishes with CA nutrient media two days prior to inoculation. The inoculated Petri dishes were sealed with parafilm and set in a closed container in complete darkness with daytime temperature of 21-23 °C for 48 hours or until fungus colonized 50-75% of the plate surface area. Eight Petri plates with mycelia were used to inoculate 200 pea plants and 10 check plants. The remaining sealed Petri plate was kept in 4 °C and used to inoculate nine Petri for the next set of inoculations. This cycle of subculturing was maintained for the duration of the experiment.

Plant Growth

A single seed was planted in 7 cm conetainers filled with Sunshine brand LC-1 professional growing mix (Sun Gro Horticulture, Canada) and watered daily. Five seeds with similar morphology were planted for each line and uniform four plants were further grown and

considered replicates. Two mist chambers were used to keep 220 conetainers; therefore, each chamber held 100 inoculated genotypes, 5 inoculated check plants and 5 non-inoculated check plants in each screening cycle. Internode type for each accession was recorded at the time of inoculation.

No fertilizer was added due to the short growing and screening period. Greenhouse conditions were maintained at 20-25 °C during daytime and 15-20 °C during nighttime. The temperature in the mist chambers were stringently regulated and daytime and nighttime temperature ranged between 19-21 °C.

Inoculation and Scoring for White Mold Resistance

Inoculation of each plant was accomplished using the agar plug technique developed by Dr. Lyndon Porter (Porter et al., 2009). An agar plug containing mycelia from the leading edge of white mold colonies grown on CA media was collected using a jumbo sized dental amalgam tool (Figure 2.2) (Safco Dental Supply Co., USA). Each Petri dish received 20 ml of media; therefore, each plug contained an equal amount of inoculum.

Figure 2.2. Dental amalgam tool was used to inoculate pea plants with *S. sclerotiorum*.

The agar plug was placed at the leaf axil of the 4th node of each 2 week old plant. The plants were held firmly at the 4th node and media was squeezed in a way that it would press around the stem. The plants were placed in a mist chamber following inoculation and conditions

were maintained at 100% relative humidity and 20 °C with complete darkness for 48 hrs. Disease lesion edges were determined as opposite points on stem where healthy stem tissue color transformed into a water-soaked color. Lesion length was measured with a digital caliper 48 hours post inoculation.

Table 2.2. Nodal transmission rating scale for white mold disease progression in *P. sativum*.

Score	Nodal transmission progress of the disease at 7 days post inoculation
4	Disease lesion did not progress from the point of inoculation at the 4th node
3.5	Disease lesion progressed from the inoculation point at the 4th node to midpoint of the internode section between 3rd and 4th node
3	Disease lesion progressed from the inoculation point at the 4th node to the 3rd node
2.5	Disease lesion progressed from the inoculation point at the 4th node to midpoint of the internode section between 2nd and 3rd node
2	Disease lesion progressed from the inoculation point at the 4th node to the 2nd node
1.5	Disease lesion progressed from the inoculation point at the 4th node to midpoint of the internode section between 1st and 2nd node
1	Disease lesion progressed from the inoculation point at 4th node to the 1st node
0.5	Disease lesion progressed from the inoculation point at 4th node to midpoint of the internode section between root system and 1st node
0	Complete plant death

After lesions were measured the plants were returned to the mist chamber. The relative humidity was lowered to 80% and light was provided at a 14:10 light:darkness photoperiod for the next five days. Seven days post inoculation nodal transmission scores were measured. Nodal transmission scores were assigned according to criteria listed in Table 2.2. Plants that remained erect two days post inoculation were noted. Susceptible pea variety 'Stirling' was used as a

check throughout the screening process. Screening the PI accessions was conducted from June to September, 2011.

Statistical Analysis

Lesion length data of repeated control plant, 'Stirling', was analyzed using PROC GLM in SAS ® 9.2 (SAS Institute Inc., USA). Levene's test for homogeneity of lesion length variance of check plants among experimental sets were analyzed to justify combining all screening sets. Histogram and descriptive statistics for the disease lesion length and nodal transmission scores were calculated using Microsoft® Office Excel 2010 SP1 (Microsoft Corporation, USA).

Results

Extensive seed and plant morphological variation was observed. Seeds of some accessions were uniform in appearance and size while others showed mixtures of seed testa colors and patterns (Figure 2.1). In some cases, seedling showed different internode types as well as plant structure. Analysis of variance for disease lesion length of the common check 'Stirling' across the ten screening sets showed no significant difference among sets ($P<0.05$). Therefore, data for all ten sets were combined. Nodal transmission scores of 'Stirling' could not be analyzed for variance among test sets since the seven day test resulted in complete death of the plants and a score of 0. Among 500 accessions in the *Pisum* core collection 15 failed to germinate or were too small for disease inoculation and were excluded from the data analysis. Among of 485 genotypes 63 were short internode type. All genotypes possessed normal leaf phenotype.

The mean disease lesion and mean nodal transmission score were based on 2-4 plants per accession. Minimum mean lesion length was 9.7 mm and maximum was 65.6 and thus produce a range of 55.9 mm. Mean, median, and mode of mean lesion length are comparable indicating

normal distribution of the data (Table 2.3). Range for nodal transmission score was 3 with minimum score of 0 and maximum of 3. Nodal transmission scores were skewed toward complete susceptibility with mode and median locating closer to complete susceptibility (Table 2.3).

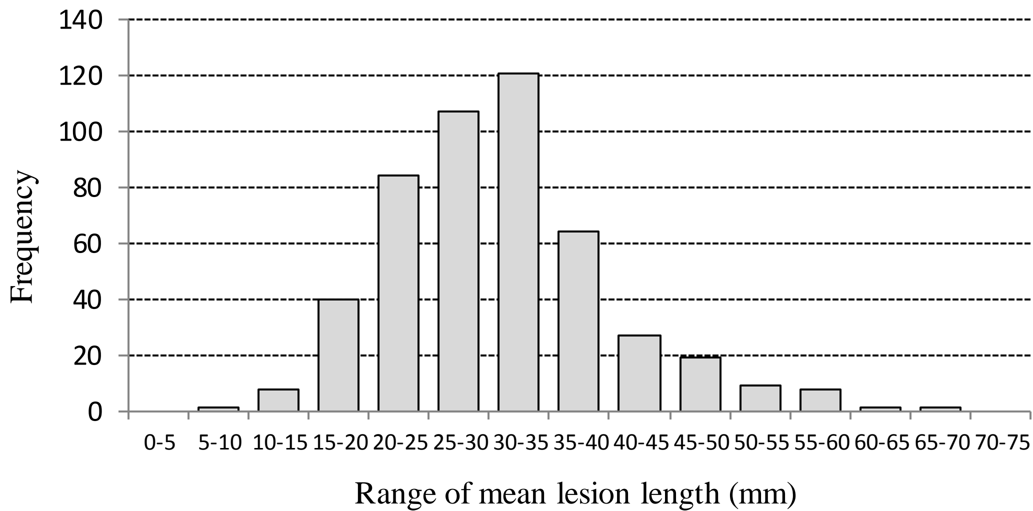


Figure 2.3. Histogram for mean lesion length recorded 48 h post inoculation with *S. sclerotiorum* for 484 pea accessions from the *Pisum* core collection.

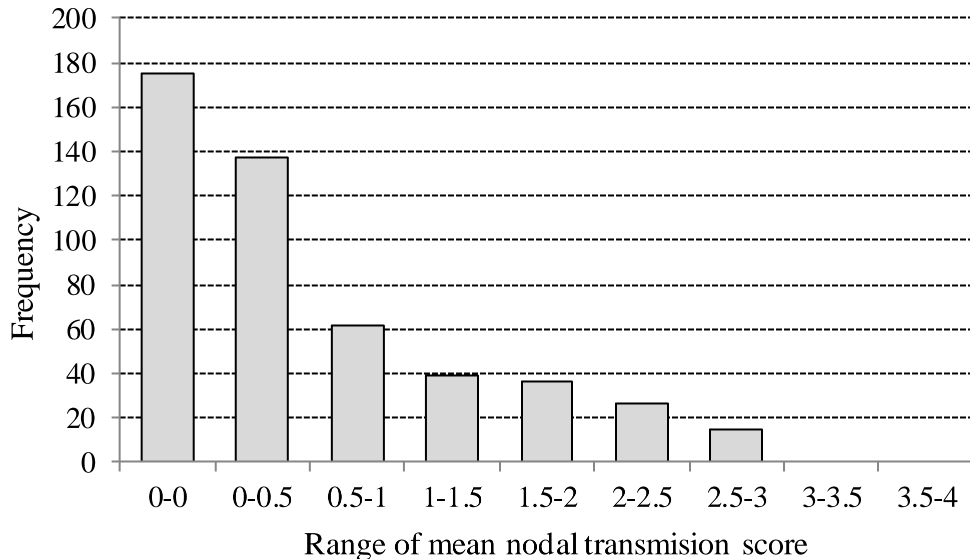


Figure 2.4. Histogram for mean nodal transmission score recorded 7 days post inoculation with *S. sclerotiorum* for 484 pea accessions from the *Pisum* core collection.

Table 2.3. Descriptive statistics of lesion length and nodal transmission score of 484 pea accessions.

Test Statistics	Lesion Length	Nodal Transmission
Mean	30.7	0.6
Median	30.1	0.3
Mode	32.8	0.0
Standard Deviation	9.2	0.8
Kurtosis	0.9	0.4
Skewness	0.7	1.2
Minimum	9.7	0.0
Maximum	65.6	3.0

All pea genotypes tested were susceptible to *S. sclerotiorum*. Sixty-four genotypes showed partial resistance to white mold based on mean disease lesion length less than 2.0 cm and/or nodal transmission score more than 2.0. The check, 'Stirling', had a mean disease lesion length of 19.8 mm and mean nodal transmission score of 0, indicating complete plant takeover by the pathogen seven days post inoculation. All accessions with short internode had nodal transmission scores below 1.0.

Table 2.4. *P. sativum* accessions that demonstrated the greatest resistance to white mold. Means are based on 3-4 plants per accession.

Accession ID	Taxonomy	Lesion length (mm)	Nodal transmission	Number of plants tested	Origin
PI155109	*P. sativum*	17.8	2.3	4	
PI210583	*P. sativum*	22.5	2.5	4	USA
PI263027	*P. sativum*	17.6	2.8	4	France
PI269797	*P. sativum*	23.4	2.5	4	England, UK
PI269802	*P. sativum*	21.6	2.6	4	UK
PI271038	*P. sativum*	17.4	2.3	4	Nepal
PI271511	*P. sativum*	24.8	2.1	4	India
PI319374	*P. sativum*	20.7	2.6	4	Mexico
PI357289	*P. sativum*	12.2	2.7	4	Former Serbia and Montenegro
PI357290	*P. sativum*	22.6	2.0	4	Former Serbia and Montenegro
PI413686	*P. sativum*	21.9	2.1	4	Hungary
PI413697	*P. sativum*	24.2	2.0	4	Hungary
PI477371	*P. sativum*	22.1	2.6	3	Denmark

Thirteen pea accessions showed the highest partial resistance to the pathogen based on criteria of mean lesion length of 25.0 mm or less and mean nodal transmission score of 2.0 or more (Table 2.4). The mean lesion length and mean nodal transmission score for each accession is given in the Appendix along with passport information of the genotypes.

Discussion

Wide morphological variation was observed in the *Pisum* core collection indicating considerable genetic diversity and variation. The results indicate presence of moderate resistance to white mold within the core collection. 422 accessions had long vine type while 63 accessions had short vines; all accessions had normal leaf type.

The seven day test for nodal transmission resulted in excessive disease pressure for all short internode type pea lines and often resulted in plants being completely overcome. Future disease screening experiments should be adjusted such that the internode length is standardized using cut-stem inoculation technique. Utilizing this method the time that is required for *S. sclerotiorum* to pass through the node can be recorded and the data would represent nodal resistance more accurately.

The lack of complete resistance to white mold in the core collection was not surprising. Complete resistance to *S. sclerotiorum* in other crops such as soybean, sunflower, and canola has not been reported (Bolton et al., 2006). *S. sclerotiorum* is a ubiquitous pathogen with a wide host range and it has many mechanisms to effectively infect and kill the host. Oxalic acid, for instance, can disrupt the host physiology in many ways such as plugging xylem tissue, interrupting stomata closure, lowering intracellular pH, programmed cell death, and sequestration of divalent cations.

It is presumed that partial resistance to the pathogen is quantitative in nature. Partial resistance in pea is expressed in two forms; through lesion expansion inhibition (LEI) and nodal transmission inhibition (NTI). Both traits have critical factors for the disease development in the field and contribution to yield. LEI is likely the result of physiological processes in the tissues exposed to white mold mycelia. This can be seen as a slowed rate of lesion progression. In the field, conditions slowing disease progression during critical weather conditions may enable plant to endure white mold attack during the pathogenicity window. Pea plants are known to produce branches in field conditions and nodal transmission inhibition may resist the pathogen to only the diseased branches.

Eight out of thirteen most resistant accessions have not been reported previously as partially resistant pea genotypes to the pathogen. These accessions were not previously available from *Pisum* core collection. Also each accession may not be genetically uniform and therefore previous screens may have missed the partially resistant accessions.

Four genotypes, PI263027, PI269802, PI319374, and PI357290 with the greatest degree of partial resistance to *S. sclerotiorum* also had the greatest root to shoot ratio (McPhee, 2005). In addition, among thirteen most partially resistant pea accession six genotypes, PI263027, PI269797, PI271038, PI319374, PI413697, and PI477371 had highest stem strength (McPhee and Muehlbauer, 1999). In other investigations high correlation of stem diameter and disease lesion inhibition was found (Blanchette and Auld, 1978).

The two traits used so far to characterize resistance may not be adequate for substantial control of white mold. Several accessions failed to break at the point of inoculation and were erect two days post inoculation. This phenotype may be beneficial to add greater disease resistance. Intact canopy of infected pea with S. *sclerotiorum* can reduce the microclimate

conditions required for white mold infestation. In addition, if plant parts fail to break at the infection point, it may reduce the nutrient available to germinating ascospores on the soil surface. The genetic component of this trait should be studied in greater detail in future experiment.

Disease reaction scores for each pea accession tested will be publicly available and searchable for other researchers interested in accessing the *Pisum* core collection germplasm as a source of white mold resistance. The results reported here are essential to designing crossing populations for the study of the inheritance and combining white mold resistance genes and transferring to adapted cultivars.

CHAPTER 3. MOLECULAR SCREENING FOR QTL ASSOCIATED WITH RESISTANCE TO WHITE MOLD IN *PISUM SATIVUM*

Introduction

Field pea (*Pisum sativum* L.) is a cool season legume grown in temperate areas of the world. Field pea seed is rich in protein, carbohydrates, minerals, and fiber. Primary uses for field pea seeds are in animal feed and to some degree in human diets (Muehlbauer et al., 1983). Seeds are used as whole, split, or made into flour (Davies et al., 1985).

Field pea is adversely affected by several fungal, bacterial and viral pathogens. Among soil-borne infectious fungi that can attack and kill pea, *Sclerotinia sclerotiorum* (Lib) de Bary is a necrotrophic, opportunistic pathogen that can infect many crops grown in the Northern Plains region. *Sclerotinia sclerotiorum* causes white mold of pea. The characteristic syndromes of the disease are mycelial growth and water soaked lesions on affected plant tissues; the disease causes widespread loss of yield and quality in pea.

Integrated pest management method is used for white mold control in pea. This method utilizes several key cultural practices. One of them is timely application of fungicides. Foliar applications of expensive fungicides applied prophylactically can control white mold. Timing of application is critical, and multiple applications may be needed (Sugha, 2002). Additionally, the environmental and human health impacts are concerns as well.

Domestication of pea resulted in a genetic bottleneck; common pattern to many crops (Tanksley and McCouch, 1997). The primary gene pool of in *Pisum* consists of five subspecies and four varieties (Xu-xiao et al., 2009). The secondary gene pool includes *P. fulvum*. There are no technical difficulties in crossing and obtaining viable hybrids; however when two species are crossed *P. fulvum* should be used as a pollen source. Pea germplasm improvement through

increasing tolerance to biotic and abiotic and agronomic traits encompasses introduction of exotic germplasm for acquiring traits of interest. Conventional breeders fully understand that success rate in process of crop improvement dependent upon genetic resources available.

Studies of host genetic variation for resistance focus on identifying and utilizing heritable components of physiological, morphological and biochemical traits that limit growth and reproduction of pathogens. Wild accessions of pea can serve as a source for such genetic variation. Currently more than 500 accessions are available from Western regional Plant Introduction System. These are cultivars, accessions of pea from 67 countries (Coyne et al., 2005).

Many agronomically important traits are characterized by quantitative inheritance. Several disease resistance traits involve QTL and have been studied previously. In one study, thirteen QTLs were detected for ascochyta blight resistance on seven linkage groups (Timmerman-Vaughan et al., 2002). In another study, two putative QTLs for resistance to *Sclerotinia* stem rot of soybean were identified on two linkage groups in a RIL population developed from a cross of partially resistance soybean PI 194639 and susceptible cultivar 'Merit' (Vuong et al., 2008). The parents in these studies were initially screened for polymorphism with available markers that were developed previously.

Marker-assisted selection (MAS) may aid in the research to find and incorporate physiological resistance into new crop cultivars. Markers tightly linked to resistance genes and QTL allow screening for genotype as a predictor of phenotype, improving the effectiveness of selection. For example, a single QTL on linkage group K of soybean was directly related to partial physiological resistance and not escape mechanism traits and could be used in MAS by breeders (Kim and Diers, 2000).

Developing disease resistant cultivars with MAS can be very effective tool. It cannot eliminate field screening for the disease but it may increase selection efficiency and save resources for breeders. Objectives of this research were to, 1) develop a genetic map of the cross, 'Lifter'/PI240515, and 2) identify and place the gene(s)/QTL controlling partial resistance to white mold in the 'Lifter'/PI240515 population.

Materials and Methods

Development of Genetic Population and Maintenance

PI240515 is an exotic pea accession identified in a 2007 greenhouse test with resistance to the *S. sclerotiorum* (Porter, 2009). The accession demonstrated a moderate level of nodal resistance and survival at seven days post inoculation. PI240515 originated from India and has long internodes, normal leaf morphology, white flowers, and yellowish green seed. 'Lifter" was developed by the USDA-ARS in cooperation with the Washington Agricultural Research Center, Pullman, WA, ant the Idaho Agricultural Experiment Station, Moscow, ID (McPhee and Muehlbauer, 2002). Lifter has a short internodes, with semi-leafless morphology, white flowers, and green seed.

A single F_1 seed was grown hydroponically and 205 F_2 seeds were selected at random to develop the mapping population. All 205 seeds were planted in the greenhouse; however, tissue samples and seeds were collected from 190 F_2 plants. The F_3 seeds were collected for disease evaluation. A single random F_3 seed was planted back toward development of recombinant inbred lines. F_4 seed was also harvested for disease evaluation.

Fungal Maintenance and Inoculation

The inoculation procedure for the F_3 and F_4 plants was carried out by collaborators at USDA-ARS in Prosser, WA. The techniques and resources that were used in maintaining *S. sclerotiorum* cultures and disease evaluation are similar or comparable to methods and resources used to screen the *Pisum* core collection at Fargo, ND and was described in Chapter 2.

The *S. sclerotiorum* isolate was collected in October, 2002 as sclerotia from pea cultivar 'Snake'. Inoculum for the experiment was collected from mycelial colonies grown on PDA media with a dental amalgam tool and placed on the leaf axil of the 4th node. The plants were placed in a mist chamber for 72 h after inoculation and maintained at 95% relative humidity. Disease lesion lengths were measured at 48 h after inoculation. Plants were assessed for survival and nodal resistance seven days post inoculation.

Survival of inoculated plants was based on whether the plants were living two weeks after inoculation. The second factor, nodal resistance, was based on the progress of the lesion down the stem of plants two weeks after inoculation. The severity values assigned as nodal resistance were assessed on a scale of 0 to 4. Nodal resistance of 0 was assigned if plant did not survive, 1 if the lesion expanded from the 4th node to the 1st node, 2 if the lesion expanded from the 4th node to the 2nd node, 3 if the lesion expanded from the 4th node to the 3rd node, and 4 if the lesion did not expand from the point where it was inoculated at the 4th node. Half-values also were assigned if the lesion stopped between the nodes.

A randomized complete block design (RCBD) with four replications was used to evaluate each pea line for disease reaction. As positive and negative controls, inoculated and non-inoculated plants of the white mold susceptible pea cultivar Bolero were included.

Pea DNA Extraction

Approximately one gram of fresh leaf tissue samples from 190 F_2 plants from the Lifter/PI240515 population were collected and freeze-dried. The dried tissue samples were transferred into 50 ml plastic tubes containing approximately 100 milligrams of silica gel and two 5 mm steel beads. The tissue samples were ground into a fine powder using a paint shaker.

Extraction buffer containing cetyltrimethylammonium bromide (CTAB) was prepared using Norm's mini mix protocol. Each tube containing ground tissue samples received 15 ml of buffer was gently mixed and heated to 65 °C for 30 minutes. Once removed from the water bath and cooled for five minutes each tube received 15 ml of chloroform/iso-amyl alcohol (24:1) solution. The solution was mixed by inverting the tubes for several times.

Once mixing was complete the samples were centrifuged at 2200 rpm for 15 minutes. The spinning process produced three distinct layers of solution that contained different fractions of plant cell parts. The top layer containing nucleic acids was pipetted into new tubes and 30 µl of RNase (10 mg/ ml) solution was added. The tubes were then placed in a 37 °C water bath for 30 minutes. Fifteen ml of chloroform/iso-amyl alcohol (24:1) solution was added to each tube, mixed and spun in the centrifuge at 2200 rpm for 15 min. The aqueous layer was transferred to new tubes and DNA in each tube was precipitated by adding cold 95% ethanol (2X amount of sample) and inverting the samples several times and leaving in 4 °C for 20 minutes. The DNA was accumulated to the bottom of the tubes after 10 minute of centrifugation at 2200 rpm. The supernatant was poured off and the pellet was washed with 20 ml of cold 70% ethanol by gently inverting the tubes for five minutes. The supernatant was removed and the pellet was dried in the fume hood.

The tubes received 1 ml of sterile Tris-EDTA (TE) buffer and were allowed to hydrate overnight. Quantification and quality evaluation of DNA from each tube was performed using a 384 Plus SpectraMax® (Sunnyvale, CA) spectrophotometer using SOFTmax® PRO 3.1 software by measuring the optical density of nucleic acids and proteins in samples. DNA samples were standardized to 100 µg/ml using TE buffer. Quality check of extracted DNA was also performed by loading 5 µl of DNA samples into a 2.5% agarose gel and run at 90 mV for two hours.

PCR and Imaging

SSR

Pisum short sequence repeats (SSR) were designed by the AgroGene Company (Moissy Cramayel, France) through an international consortium and used to develop the *P. sativum* consensus genetic map reported by Loridon et al. (2005). The SSR PCR primer reactions were performed in 15 µl volumes containing 1.5 µl 10X standard PCR buffer, 0.9 µl of 25 mM $MgCl_2$, 3 µl of 1 mM dNTPs, 0.05 µl of 100 µM of the forward and reverse primers, 0.1 to 0.5 units of Taq DNA polymerase, and 2 µl of ~100 µg/ml genomic DNA.

The optimum annealing temperature for each primer pair was determined by testing parents using PCR at three different annealing temperature settings: 50 °C, 55 °C and 60 °C. The recommended annealing temperature for each primer set was compared with that from the manufacturer to determine the optimal conditions for population screening.

After the optimum annealing temperature was determined for the parents, F_2 population DNA was amplified using Veriti thermocycler (Applied Biosystems Inc., CA) or Life Pro thermocycler (Bioer Technologies, LTD) programmed for 35 cycles of 40 s denaturation at 94

°C, 55 s at the optimum annealing temperature, and 60 s extension at 72 °C. The final extension step was followed by 7 min of finalizing step at 72 °C and products were maintained at 4 °C.

CAPS

Cleaved amplified polymorphic sequence (CAPS) based markers were also used for genotyping purposes. Oligomer primers were purchased from Eurofins MWG Operon (Ebersberg, Germany). The CAPS PCR primer reactions were performed in 15 µl volumes containing 1.5 µl 10X standard PCR buffer, 0.9 µl of 25 mM $MgCl_2$, 3 µl of 1 mM dNTPs, 0.05 µl of 100 µM of the forward and reverse primers, 0.1 to 0.5 units of Taq DNA polymerase, and 2 µl of ~100 µg/ml genomic DNA.

The PCR amplification profile for CAPS used the same cycle used for the SSR reactions. PCR reactions received 0.1 µl of restriction enzyme (New England Biolabs Inc, Ipswich, MA), 2 µl of enzyme buffer (Sigma LLC, St. Lois, MO) and 3 µl of water. The PCR plate then was placed in 37 °C water bath for 2 hours.

RAPD

Random amplified polymorphic DNA (RAPD) markers based on 10-mer primers obtained from Qiagen Inc (Valencia, CA). The RAPD PCR primer reactions were performed in 15 µl volumes containing 1.5 µl 10X standard PCR buffer, 0.9 µl of 25 mM $MgCl_2$, 3 µl of 1 mM dNTPs, 0.05 µl of 100 µM of primer, 0.5 units of Taq DNA polymerase, and 2 µl of ~100 µg/ml genomic DNA.

The PCR procedure was performed using Veriti thermocycler (Applied Biosystems Inc., CA) or Life Pro thermocycler (Bioer Technologies, LTD) using the PCR profile that was set for 40 cycles of a 20 s at 94 °C, 60 s at 35 °C, and 90 s at 72 °C. The final extension step was followed by 7 minutes at 72 °C and products were maintained at 4 °C after that.

Imaging

PCR products were separated on a 2.5% agarose gel system or 6.5% non-denaturing polyacrylamide gel system and stained with ethidium bromide. Visualization was performed using Bio-Rad GelDoc 2000 UV transilluminator digital imaging system (Bio-Rad laboratories, CA). Primer pairs were scored by amplification of products matching the expected size bands observed in the parents. In co-dominant markers, amplicon bands were scored "A" for the Lifter allele, or "B" for the parent PI240515 allele, or "H" for the heterozygous condition. In dominant markers, amplicon bands were scored "A" for the Lifter allele or "C" for the parent PI240515 allele and the heterozygous condition or "B" for the parent PI240515 allele or "D" for parent 'Lifter' allele and the heterozygous genotype.

Statistical Analysis and Linkage Map Construction

Scores for disease lesion length and nodal transmission scores for F_3 and F_4 lines were analyzed for normality using Kolmogorov-Smirnov test. Descriptive statistics were obtained using Microsoft Excel 2010, SP1 (Microsoft Corporation, USA). Chi-Square (χ^2), linkage groups, and cumulative recombination frequencies were identified using JoinMap® 4, (Plant Research International B.V. and Kyazma B.V.) (Van Ooijen 2006). Linkage groupings were established using linkage parameter with LOD score of 4.0. Placing markers on linkage groups were performed using maximum likelihood (ML) algorithm using software default settings.

Windows QTL Cartographer Version 2.5, Statistical Genetics, North Carolina State University, NC (Wang et al. 2010) was used for single marker analysis (SMA), interval mapping (IM), and composite interval mapping (CIM) analysis with LOD threshold level estimated with 500 ($P<0.05$) for each trait. Interval mapping and composite interval mapping with walking speed of 1 cM to 3 cM was used to locate putative QTL.

Results

Linkage Map

Two hundred and thirty-nine SSR markers were screened against the two parents at three different annealing temperatures. Optimal annealing temperatures for each primer pair and expected band sizes were identified based on the parental screen. Polymorphic primers in the parents were amplified in the total population of one hundred ninety F_2 progeny. Twenty-nine primer pairs failed to amplify and one hundred sixteen primer pairs were monomorphic. Seventy microsatellite markers were used for mapping with three primers amplifying two loci, and the remaining sixty-seven contributing a single polymorphic locus for a total of seventy-three polymorphic loci (Table 3.1). About 40% of the SSRs primers and 46% of all primers were polymorphic with at least one usable locus. Similar to microsatellites, CAPS and RAPD markers were initially screened against the parents to determine polymorphism and band sizes. CAPSs and RAPDs also provided high levels of polymorphism.

Table 3.1. Summary of molecular markers used to create a linkage map for the *P. sativum* based on the cross Lifter/PI240515.

Type of Marker	Primers					
	Tested	Failed	Monomorphic	Polymorphic	Used	Total Bands
	(No.)					
SSR	239	29	116	94	70	73
CAPS	24	6	12	6	3	3
RAPD	146	8	48	90	1	2
Total	409	43	176	190	74	78

One individual out of 190 F_2 individuals was omitted from linkage group analysis due to 96% similarity with another individual. Seventy-eight marker loci were tested for goodness-of-fit for allelic segregation to the expected Mendelian segregation ratio of 1:2:1 using the χ^2 test

(*P*<0.05). Three loci were omitted from linkage analysis due to distortion. Eight markers remained unlinked to any linkage group.

Nine linkage groups (LG) were generated using a linkage LOD with minimum score of 4.0 and markers were placed using maximum likelihood mapping default settings. Nine LGs spanned 734 cM (Figure 3.1). Based on collinearity of markers on nine LGs and consensus Pisum map (Loridon et al, 2005) LG II (b) and LG V (b) are part of LG II and LG V of pea.

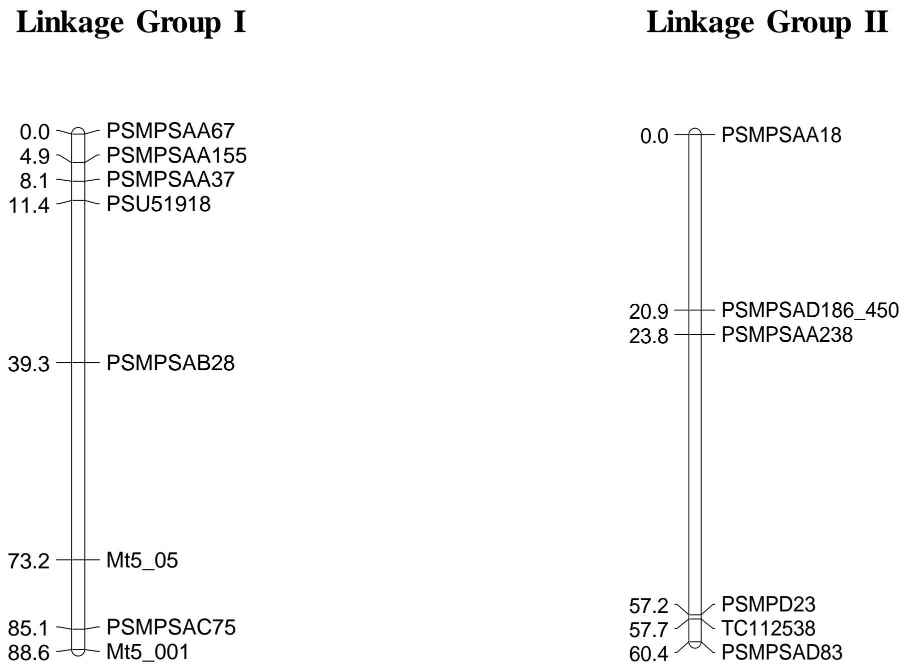
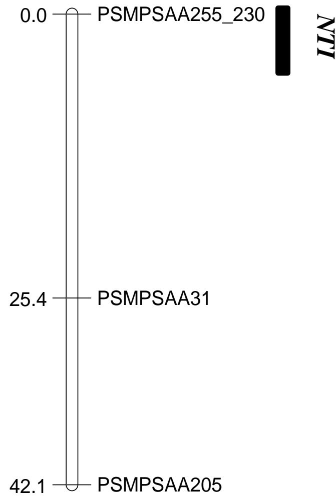
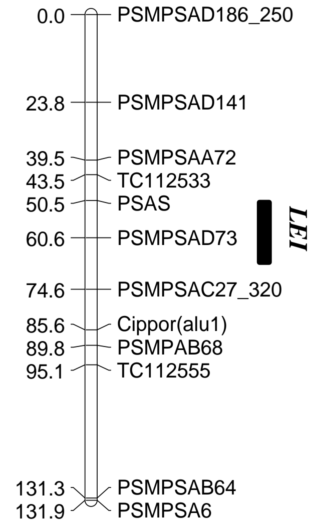


Figure 3. 1. Linkage map created with SSR, CAPS, and RAPD markers from 189 individuals in Lifter/PI240515 F2 population. Markers name is shown on the right side of LG with distance in cM on the left. Putative QTL for LEI and NTI are displayed as crossbar to the right of the flanking markers.

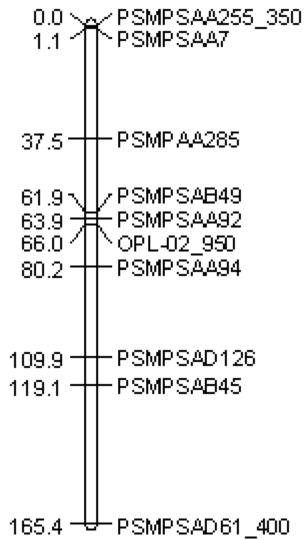
Linkage Group II (b)



Linkage Group III



Linkage Group IV



Linkage group V

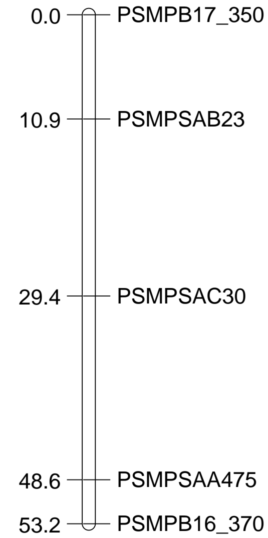
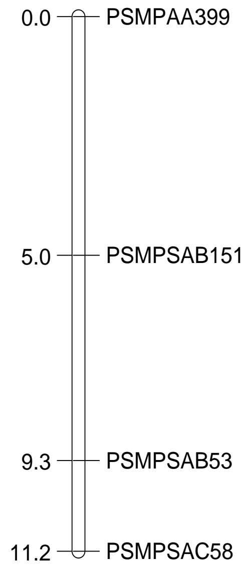


Figure 3. 1. (Continued)

Linkage Group V (b)



Linkage Group VI

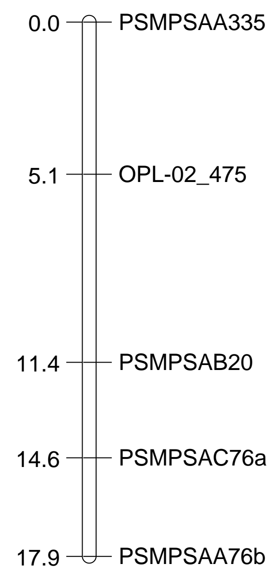


Figure 3. 1. (Continued)

Linkage Group VII

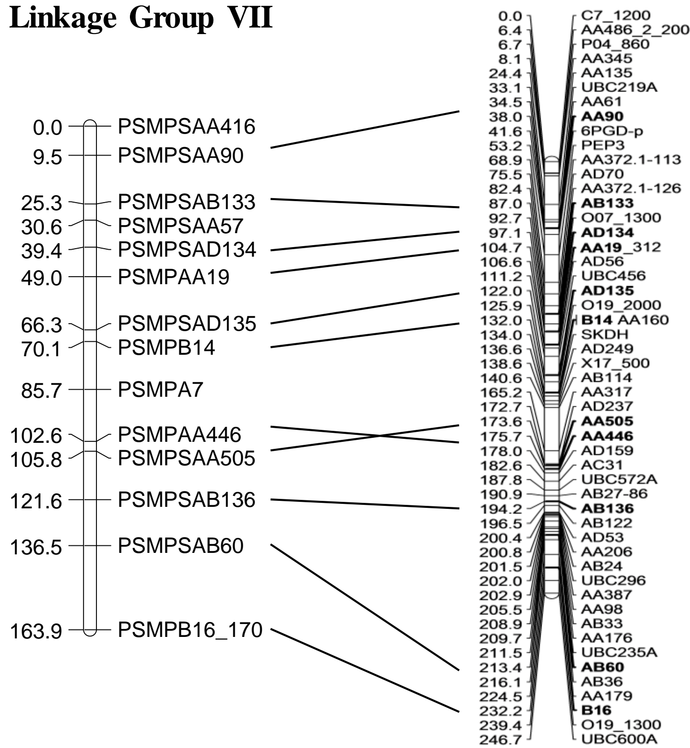


Figure 3. 2. LGVII developed from Lifter/PI240515 F_2 map (right) and collinearity of the mapped markers to LGVII of established pea consensus map (left) from Burstin et al., (2006).

Resistance QTL

Interval mapping (IM) and composite interval mapping (CIM) analysis with LOD threshold level calculated based on 500 permutations (*P*<0.05) revealed a single QTL conferring LEI on LGIII. QTL associated with NTI was identified only with CIM analysis. A single peak was identified in LGII(b).

The closest marker associated with the QTL responsible for LEI was located toward the middle of the LGIII, close to PSMPSAD73, a microsatellite marker, and phenotypic variation explained by this QTL was 34.1% (Figure 3.3).

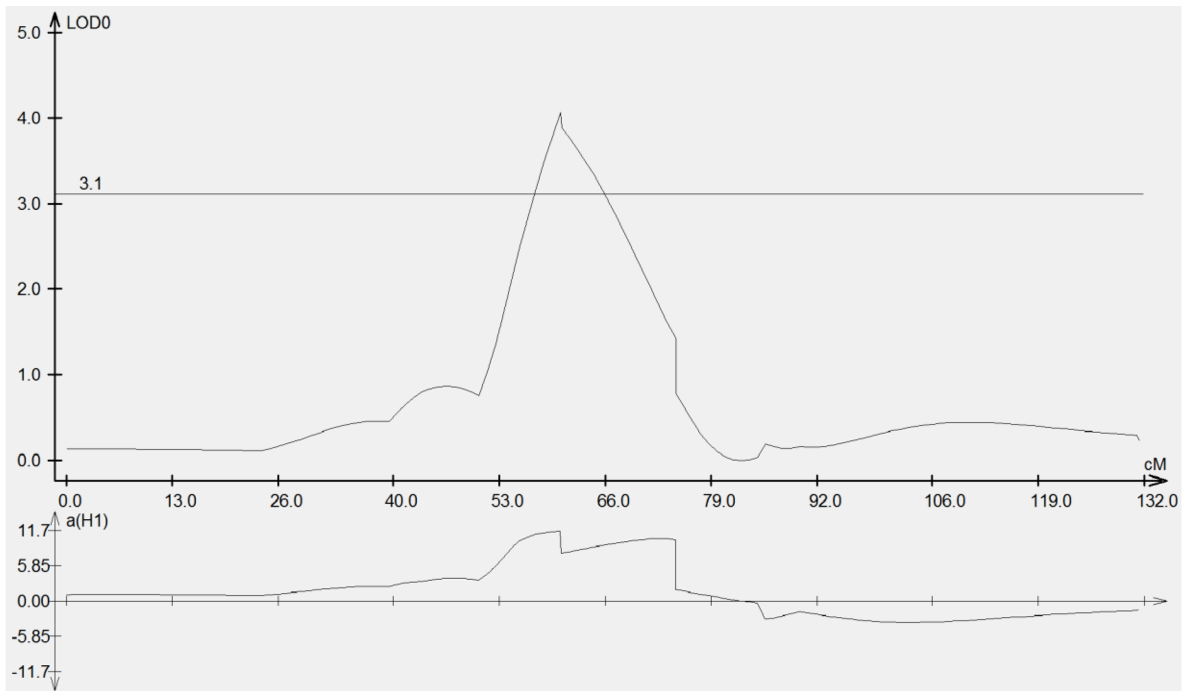


Figure 3. 3. Location of QTL on LGIII conferring LEI in Lifter/PI240515 F_2 population. LOD scores are from composite interval mapping and scores above the threshold level of 3.1 determined with 500 permutations at P<0.05.

The closest marker associated with QTL on LGII(b) responsible for NTI based partial resistance was PSMPSAA255_230 and phenotypic variation explained by this QTL is 2.5% (Figure 3.4). Since the end of the linkage group where putative QTL for NTI is located can be

joined to the rest of the LGII, additional markers in this region may change the QTL location and phenotypic contribution.

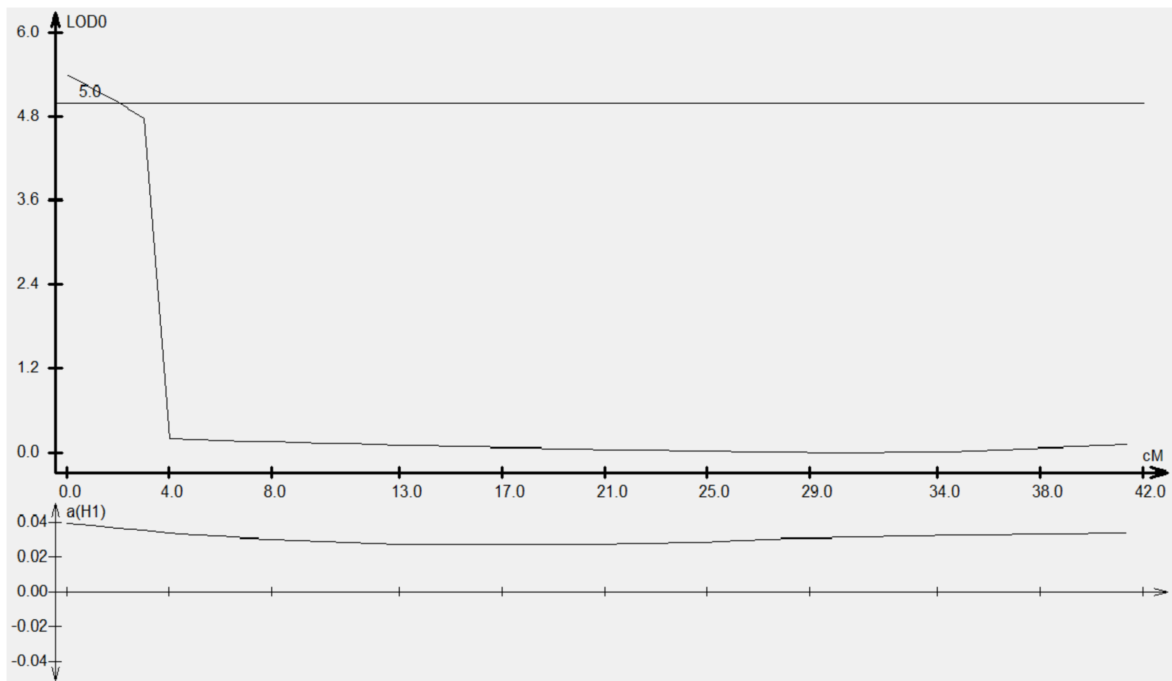


Figure 3. 4. Location of QTL on LGII(b)conferring NTI in Lifter/PI240515 F_2 population. LOD scores are from composite interval mapping and scores above the threshold level of 5.0 determined with 500 permutations at P<0.05.

Discussion

The two parents, Lifter and PI241505, are considerably diverse in terms of morphology and geographic origin; therefore, greater polymorphism was expected. PI240515 was collected in India, whereas Lifter was developed in the US Pacific Northwest as a locally adapted variety. Morphologically the two parents are very different in appearance and growth habit. PI240515 has a long vine type with greater vegetative mass, longer pods, large, yellow-brown colored seeds, whereas Lifter is shorter in stature, has shorter pods, and green cotyledons. In addition, *P. sativum* being a self-pollinating species contributes to fairly high levels of genetic polymorphism

among different genetic populations (Hamrick and Godt, 1996). In our tests nearly 40% of SSR markers and 46% of all markers tested showed polymorphism.

Several genetic maps of *P. sativum* using different molecular marker systems have been developed spanning from 565 cM (Knox and Ellis, 2002) up to 2300 cM (Hall et al., 1997). The current map of Lifter/PI240515 consisting 67 loci on nine linkage groups spans 734 cM. While additional markers would increase the map length, the discrepancy in map length is influenced by the population that was used for mapping. Knox and Ellis reported that excess heterozygosity in pea recombinant inbred populations contribute to genetic map expansion (Knox and Ellis, 2002). Future genetic mapping of Lifter/PI240515 using RIL population can result in a larger map.

There are nine LGs in the current map and only linkage group six partially covered the corresponding chromosome developed for the pea consensus map while other LGs have covered most of the corresponding consensus linkage groups (Loridon et al, 2005). The markers on each LGs and the consensus map LGs were collienear and used to identify the truncated LGII(b) and LGV(b).

Phenotypic observation of disease reaction in Pisum core collection and F3, and F4 mapping population of Lifter/PI240515 indicates the quantitative nature of the white mold resistance in pea. However only of single QTL for each trait were identified. The relatively sparse markers on such a large genome of pea may be the reason. Future genetic mapping of Lifter/PI240515 using RIL population with more markers will enable to identify additional QTL.

The importance of unbiased phenotypic evaluation technique is crucial in identification of valid QTL. The morphological difference of internode length of individuals within a mapping population creates a potential for nodes to interfere with disease progression may impact and bias

disease lesion expansion, especially in short internode types. The possibility that the disease progression is confounded by internode length should be accounted for in future experiments. Additionally, disease evaluation technique will be modified to better estimate resistance level. Considering these findings in the future experiment the population can be divided into short and long vine type for valid disease screening and statistical analysis.

Currently no QTL have been reported in pea conferring resistance to s. sclerotiorum in order for comparison. Therefore validation of identified QTL and presence of additional QTL for LEI and NTI will be further investigated in recombinant inbred line population developed from the same cross. In addition there is additional population, Medora/PI169306 that will be investigated using same phenotyping techniques. Having RIL populations from these crosses will enable phenotyping in the field as well.

CHAPTER 4. OVERALL CONCLUSION

Field pea (*Pisum sativum* L.) is a wholesome legume, high in fiber and protein and used for human consumption and as animal feed worldwide. In 2010, North Dakota led the nation in dry pea production, with total production worth more than $98 million (NASS/USDA, 2011). Among many biotic stresses that adversely affect pea yield and seed quality, white mold can cause significant losses and is found all around the world.

The fungus *Sclerotinia sclerotiorum* (Lib.) de Bary is responsible for white mold disease that is also known by other names such as cottony rot, watery soft rot, crown rot, etc. (Bolton et al., 2006). Conventional control of the disease has been difficult due to a large number of major crops serving as a host plants within major crops and relatively prolonged survival of the pathogen in the soil (Blanchette and Auld, 1978).

In pea and other major susceptible crops, plant accessions with complete physiological resistance to the pathogen have not been found (Bolton et al., 2006). However, improving partial resistance to white mold disease in these crops is still a feasible solution for development of resistant new cultivars as an economically viable control method. At this point, novel disease screening methods and pea accessions with partial resistance to the pathogen have been discovered. Developing a linkage map of QTL responsible for partial resistance will reveal the genetic nature of the disease and is the primary goal of this study.

Greenhouse screening of pea accessions and molecular screening of an F_2 mapping population were conducted to: 1) identify pea accessions with greatest resistance to white mold; and 2) create genetic map based on F_2 population developed from Lifter/PI240515 cross; and 3) identify QTL responsible for partial resistance to white mold in pea.

During greenhouse experiment wide morphological variation was observed in the *Pisum* core collection indicating considerable genetic diversity and variation. Out of 484 accessions screened, 421 accessions had long vine type while 63 accessions had short vines; all accessions had normal leaf type. All pea accessions that were screened developed disease symptoms with varying degree. Screening pea accessions identified several genotypes with moderate resistance to white mold within the core collection.

49 accessions had lesion expansion inhibition (LEI) based on lesion length of 2 cm or less. 41 accessions expressed partial resistance of nodal transmission inhibition (NTI) based on disease inhibition less than two nodes from the inoculation point. Thirteen accessions originating from nine countries demonstrated the greatest resistance and had both traits. Eight out of thirteen most resistant accessions have not been reported previously as partially resistant pea genotypes to the pathogen. These accessions were not previously available from *Pisum* core collection. Also each accession may not be genetically uniform and therefore previous screens may have missed the partially resistant accessions.

Disease reaction scores for lesion length were distributed comparably to normal curve. Nodal transmission scores were skewed toward complete susceptibility. The seven day test for nodal transmission resulted in excessive disease pressure for all short internode type pea lines and often resulted in plants being completely overcome. Future disease screening experiments should be adjusted such that the internode length is standardized using cut-stem inoculation technique. Utilizing this method the time that is required for *S. sclerotiorum* to pass through the node can be recorded and the data would represent nodal resistance more accurately.

The lack of complete resistance to white mold in the core collection was not surprising. Complete resistance to *S. sclerotiorum* in other crops such as soybean, sunflower, and canola has

not been reported (Bolton et al., 2006). *S. sclerotiorum* is a ubiquitous pathogen with a wide host range and it has many mechanisms to effectively infect and kill the host. Oxalic acid, for instance, can disrupt the host physiology in many ways such as plugging xylem tissue, interrupting stomata closure, lowering intracellular pH, programmed cell death, and sequestration of divalent cations.

Disease reaction scores for each pea accession tested will be publicly available and searchable for other researchers interested in accessing the *Pisum* core collection germplasm as a source of white mold resistance. The results reported here are essential to designing crossing populations for the study of the inheritance and combining white mold resistance genes and transferring to adapted cultivars.

F_2 population based on Lifter/PI240515, spanned 734 cM and consisted of nine linkage groups (LG). Since *P. sativum* has only seven LGs (n=7) two LGs LGII and LGV had truncated LGII(b) and LGV(b) based on alignment of previously mapped SSR markers on established maps. Future genetic mapping of Lifter/PI240515 using RIL population and more markers will result in a larger map with fewer LGs. Out of nine LGs in the current map only LGVI partially covered the corresponding LG developed for the pea consensus map while other LGs have covered most of the corresponding consensus linkage groups (Loridon et al, 2005).

Quantitative nature of the white mold resistance in pea can be observed from the distribution phenotypic data of disease reaction in *Pisum* core collection and F_3, and F_4 mapping population of Lifter/PI240515. Several QTL was expected to be identified. Identification of single QTL for each trait might be due to relatively uneven and sparse distribution of markers on Lifter/PI240515 genome. Future genetic mapping of Lifter/PI240515 and Medora/PI169306 using RIL population with more saturated markers will enable to identify additional QTL.

Thus far no QTL have been reported in pea conferring resistance to *S. sclerotiorum* in order for comparison. Identified QTL in Lifter/PI240515 population and presence of additional QTL for LEI and NTI will be further investigated in recombinant inbred line population developed from the same cross and Medora/PI169306 that will be investigated using similar phenotyping and genotyping techniques. Having RIL populations from these crosses will enable phenotyping in the field to create additional data in uncontrolled environment that can be used in QTL analysis.

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APPENDIX

P. sativum passport data and _S. sclerotiorum_ disease reaction scores

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
1	PI102887	*P. sativum*	29.8	0.0		No. 7
2	PI102888	*P. sativum*	38.8	0.0		No. 8
3	PI103058	*P. sativum*	19.1	1.3		Pai Wan Tou
4	PI103709	*P. sativum*	31.3	0.4		G 11740
5	PI109866	*P. sativum*	41.8	0.7	Venezuela	Arvejas Verdes
6	PI113368	*P. sativum*	32.8	0.4	China, Nei Monggol	No. 338 (22)
7	PI116056	*P. sativum*	37.3	2.2	India	Matar
8	PI116843	*P. sativum*	30.7	0.6	Pakistan	Mattar
9	PI116844	*P. sativum*	29.0	2.6	Pakistan	Mattar
10	PI116944	*P. sativum*	33.7	0.3	Afghanistan	Moshong
11	PI117264	*P. sativum*	33.4	1.5	Turkey	No. 215
12	PI117998	*P. sativum*	36.4	2.8	Brazil	Ervilha Torta Flor R
13	PI118501	*P. sativum*	55.6	1.5	Brazil	Ervilha Branca
14	PI120623	*P. sativum*	24.2	0.9	Turkey	No. 545
15	PI120630	*P. sativum*	26.0	2.4	Turkey	No. 3257
16	PI121352	*P. sativum*	44.7	2.1	India	No. 1891
17	PI121976	*P. sativum*	34.8	0.0	India	Shandil
18	PI124478	*P. sativum*	32.3	3.0	Pakistan	Matar
19	PI124479	*P. sativum*	19.6	1.4	Pakistan	Matar
20	PI124595	*P. sativum*	18.1	0.5	Pakistan	No. 2894
21	PI125672	*P. sativum*	26.2	0.5	Austria	G 1696
22	PI125673	*P. sativum*	22.4	0.3	Austria	G 1697
23	PI125839	*P. sativum*	37.0	0.0	Afghanistan	Mizhik
24	PI125840	*P. sativum*	28.0	0.0	Afghanistan	Moshong
25	PI134271	*P. sativum*	36.2	1.3	Afghanistan	No. 10
26	PI137118	*P. sativum*	25.7	0.0		Chinese Purple
27	PI137119	*P. sativum*	24.4	0.0		Gray's
28	PI138945	*P. sativum*	34.3	0.0	Iran	Kudi
29	PI140295	*P. sativum*	25.8	1.3	Iran	No. 6459
30	PI140296	*P. sativum*	24.4	0.0	Iran	No. 6182
31	PI140298	*P. sativum*	25.5	1.7	Iran	No. 6618
32	PI141966	*P. sativum*	25.5	0.0	China, Heilongjiang	G 6728
33	PI142775	*P. sativum*	32.8	1.7		Alberga
34	PI142777	*P. sativum*	24.8	1.4		G 7086
35	PI143485	*P. sativum*	32.6	2.2		G 24180
36	PI155109	*P. sativum*	17.8	2.3	Rhode Island, US	G 12111

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
37	PI156647	*P. sativum*	26.5	0.0	Ethiopia	G 24184
39	PI162909	*P. sativum*	22.7	1.6	Paraguay	L.P. No. 4
40	PI163125	*P. sativum*	27.1	1.8	India	Matar
41	PI163126	*P. sativum*	24.3	0.9	India	Matar
42	PI163129	*P. sativum*	22.5	0.3	India	Matar
43	PI163130	*P. sativum*	22.3	0.5	India	Matar
44	PI164182	*P. sativum*	28.0	0.9	India	Matar
45	PI164548	*P. sativum*	29.7	0.6	India, Madhya Pradesh	Matar
46	PI164614	*P. sativum*	24.5	0.3	India	Patani
47	PI164779	*P. sativum*	34.2	1.5	India	Matar
48	PI164971	*P. sativum*	32.5	2.3	Turkey	No. 17
49	PI164972	*P. sativum*	31.0	2.1	Turkey	No. 48
50	PI165949	*P. sativum*	25.6	0.6	India	Matar
51	PI166084	*P. sativum*	30.9	0.3	India	Matar
52	PI166142	*P. sativum*	30.9	0.5	Nepal	No. 9688
53	PI166159	*P. sativum*	26.6	0.0	Nepal	No.9705
54	PI167273	*P. sativum*	31.7	0.5	Turkey	Bezelya
55	PI169603	*P. sativum*	28.1	1.3	Turkey	No. 2187
56	PI169608	*P. sativum*	35.1	0.4	Turkey	Bezelya Yesil
57	PI171810	*P. sativum*	21.9	1.9		No. 6754
58	PI171811	*P. sativum*	26.8	0.1	Turkey	Victoria
59	PI171814	*P. sativum*	25.2	0.5	Turkey	No. 7093
60	PI172339	*P. sativum*	22.0	0.0	Netherlands	Mansholt Pluk
61	PI173840	*P. sativum*	38.1	1.1	India	Matar
62	PI174320	*P. sativum*	31.5	2.5	Turkey	Sultani
63	PI174921	*P. sativum*	27.9	0.0	Nepal	Kalaon
64	PI174925	*P. sativum*	26.1	1.8	India	Matar
65	PI175228	*P. sativum*	33.7	0.5	China, Xizang	Shratma
66	PI175231	*P. sativum*	33.0	0.3	Nepal	Kolung
67	PI175232	*P. sativum*	32.0	0.9	India	Matar
68	PI179449	*P. sativum*	33.5	0.0	Turkey	Kulur
69	PI179450	*P. sativum*	31.7	1.8	Syria	No. 9747
70	PI179451	*P. sativum*	34.5	0.5	Syria	No. 9749
71	PI179452	*P. sativum*	31.0	1.3	Syria	Senateur
72	PI179453	*P. sativum*	23.3	1.2	Iraq	No. 9875
73	PI179459	*P. sativum*	31.3	2.0	Turkey	No. 10072
74	PI179722	*P. sativum*	27.6	0.5	India	No. 10898
75	PI179970	*P. sativum*	22.6	0.0	India	Matar
76	PI180329	*P. sativum*	31.9	1.3	India	Watana

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
77	PI180471	*P. sativum*	36.7	0.0	Turkey	Matar
78	PI180692	*P. sativum*	32.1	1.0	Germany	Hohenheimer Green Victoria
79	PI180693	*P. sativum*	54.3	0.8	Germany	Hohenheimer Pink-Flowered
80	PI180695	*P. sativum*	34.0	0.0	Germany	Lucienhofer
81	PI180696	*P. sativum*	16.8	1.9	Germany	Mahndorfer Victoria
82	PI180699	*P. sativum*	41.1	1.7	Germany	Rimpaus Green Victoria
83	PI180702	*P. sativum*	48.9	0.4	Germany	Strengs Weihenstephaner Felderbse
84	PI181799	*P. sativum*	29.4	1.7	Lebanon	No. 9901
85	PI181800	*P. sativum*	21.9	0.0	Syria	Kurul
86	PI181801	*P. sativum*	28.2	2.6	Syria	No. 9922
87	PI181958	*P. sativum*	26.5	0.0		Homs No. 334
88	PI183467	*P. sativum*	35.5	0.0	India	Matar
89	PI184128	*P. sativum*	30.2	2.7		No. 304
90	PI184129	*P. sativum*	33.4	0.3		No. 305
91	PI184130	*P. sativum*	20.2	0.0		No. 309
92	PI184131	*P. sativum*	20.9	0.0		No. 310
93	PI184784	*P. sativum*	33.0	1.8	Guinea	G 1774
94	PI188698	*P. sativum*	22.1	0.6	Nigeria	G 7184
95	PI189171	*P. sativum*	18.8	0.0		Gruno Rosyn
96	PI193578	*P. sativum*	34.5	0.1	Ethiopia	No. 8508
97	PI193584	*P. sativum*	31.2	0.0	Ethiopia	No. 8596
98	PI193590	*P. sativum*	35.1	0.0	Ethiopia	No. 8736
99	PI194348	*P. sativum*	27.6	0.2	Ethiopia	No. 9104
100	PI195020	*P. sativum*	28.6	0.0	Ethiopia	No. 9292
101	PI195404	*P. sativum*	27.6	0.0	Guatemala	Quezaltenango
102	PI195631	*P. sativum*	29.8	0.0	Ethiopia	No. 9886
103	PI196018	*P. sativum*	42.4	0.0	Ethiopia	No. 9713
104	PI196877	*P. sativum*	39.6	0.0	Ethiopia	No. 10026
105	PI197044	*P. sativum*	31.4	0.7	Honduras	No. 3005
106	PI197450	*P. sativum*	28.5	0.3	Ethiopia	No. 10207
107	PI197990	*P. sativum*	39.5	1.5	Netherlands	Vinco
108	PI198072	*P. sativum*	38.6	0.3	Sweden	Brioart
109	PI198073	*P. sativum*	26.3	0.0	Sweden	Saloart
110	PI198074	*P. sativum*	27.0	0.0	Sweden	Gorsdagsart III
111	PI198735	*P. sativum*	33.2	0.0		G 6748

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
112	PI200755	*P. sativum*	37.9	0.5	Guatemala	No. 3498
113	PI201390	*P. sativum*	31.0	0.0	Mexico	No. 3153
114	PI203064	*P. sativum*	30.6	0.0		G 6820
115	PI203066	*P. sativum*	31.6	0.0		G 6822
116	PI203067	*P. sativum*	25.2	0.8		G 6823
117	PI203068	*P. sativum*	28.3	0.6		G 6824
118	PI203069	*P. sativum*	35.2	0.8		G 6825
119	PI203944	*P. sativum*	37.8	0.0	Mexico	No. 12301
120	PI204306	*P. sativum*	30.5	0.4	Australia, Victoria	Dunn
121	PI204307	*P. sativum*	27.2	0.0	Australia, Victoria	White Brunswick
122	PI205216	*P. sativum*	26.8	0.3	Turkey	No. 1109
123	PI206006	*P. sativum*	34.5	2.1	Sweden	Debut 149
124	PI206838	*P. sativum*	27.1	1.0	US, Wisconsin	Everbearing
125	PI206845	*P. sativum*	33.9	0.0	US, Wisconsin	Ruhm Von Cassel
126	PI206848	*P. sativum*	36.5	0.0	US, Wisconsin	Quarante Deux De Sarcelle
127	PI206861	*P. sativum*	29.2	0.0	US, Wisconsin	Carters Market Gardener
128	PI207508	*P. sativum*	40.5	1.6	Afghanistan	No. 12616
129	PI209507	*P. sativum*	28.2	2.2	Costa Rica	No. 3843
130	PI210558	*P. sativum*	27.1	1.4	China	No. S-653
131	PI210561	*P. sativum*	36.2	0.2	Former Soviet Union	No. S-688
132	PI210568	*P. sativum*	32.8	1.9	Finland	Kelina
133	PI210569	*P. sativum*	31.2	1.4	Finland	Sivikka
134	PI210570	*P. sativum*	33.6	0.4	Finland	Kauviato
135	PI210571	*P. sativum*	30.4	0.0	Finland	Flo
136	PI210583	*P. sativum*	22.5	2.5	US. Iowa	Rogers Ace
137	PI212031	*P. sativum*	28.0	0.0	Iran, Tehran	No. 4
138	PI212917	*P. sativum*	30.2	2.7	India	Vatana Matar
139	PI215766	*P. sativum*	39.2	0.0	Peru	Alrecyos
140	PI216045	*P. sativum*	38.8	0.3	India	No. 13748
141	PI219705	*P. sativum*	30.3	0.0	Pakistan	Mutta
142	PI220174	*P. sativum*	30.1	2.6	Afghanistan	No. 13
143	PI220189	*P. sativum*	40.3	0.0	Afghanistan	Moshong
144	PI221697	*P. sativum*	31.7	2.8		No. 12
145	PI222071	*P. sativum*	35.1	2.8	Afghanistan	Moshong
146	PI222117	*P. sativum*	34.9	0.3	Afghanistan	Moshong
147	PI223526	*P. sativum*	30.8	0.0	Afghanistan	Moshong
148	PI223527	*P. sativum*	33.6	0.0	Afghanistan	Lobia

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
149	PI226561	*P. sativum*	28.3	0.3	Ethiopia	No. 359
150	PI226564	*P. sativum*	27.4	0.4	Ethiopia	No. 403
151	PI227258	*P. sativum*	28.5	0.1	Iran	No. 14918
152	PI227457	*P. sativum*	32.3	0.0	Iran	Sangakh
153	PI229538	*P. sativum*	29.6	1.5	Iran	No. 15381
154	PI236492	*P. sativum*	26.6	0.0	US, Iowa	Lamprecht #368
155	PI240515	*P. sativum*	24.6	1.3	India, Delhi	Kanawari
156	PI240516	*P. sativum*	21.2	0.8	India, Delhi	Lucknow Boniya
157	PI240518	*P. sativum*	18.0	0.3	India, Delhi	N. P. 29
158	PI241593	*P. sativum*	46.8	2.1	Taiwan	G 6571
159	PI242027	*P. sativum* spp. *sativum*	31.7	0.3	Denmark	G 11764
160	PI242028	*P. sativum*	34.7	1.8	Denmark	
161	PI244054	*P. sativum*	32.7	0.0	Yemen	No. 13
162	PI244055	*P. sativum*	33.3	0.3	Yemen	No. 8
163	PI244093	*P. sativum*	22.3	0.3	Netherlands	Apollo
164	PI244095	*P. sativum*	12.2	1.0	Netherlands	Aureool
165	PI244107	*P. sativum*	23.8	0.8	Netherlands	Censeur
166	PI244121	*P. sativum*	34.0	2.1	Netherlands	Crescent
167	PI244150	*P. sativum*	21.1	0.0	Netherlands	Goldkonigin
168	PI244175	*P. sativum*	9.7	0.3	Netherlands	Lage Smeltpeul
169	PI244191	*P. sativum*	33.8	0.3	Netherlands	Morgenster
170	PI248181	*P. sativum*	38.3	0.0	Rwanda	Col. No. 23171
171	PI249645	*P. sativum*	30.1	0.0		B.R. 178
172	PI249647	*P. sativum*	15.4	0.5		Table 19
173	PI250438	*P. sativum*	28.3	0.4	Czech Republic, Central Bo	Libochovicky Rany
174	PI250439	*P. sativum*	25.4	0.4	Czech Republic, Central Bo	Konservova Kralovna (CSR)
175	PI250440	*P. sativum*	21.3	0.6	Czech Republic, Central Bo	Karlovna Konservy
176	PI250441	*P. sativum*	26.2	0.4	Czech Republic, Central Bo	Prebohaty
177	PI250442	*P. sativum*	26.5	0.0	Czech Republic, Central Bo	Heinrichuv Rany
178	PI250444	*P. sativum*	16.1	0.1	Czech Republic, Central Bo	Zazrak Z Kelvedonu
179	PI250446	*P. sativum*	13.1	0.4	Czech Republic, Central Bo	Libochovicky Urodny
180	PI250447	*P. sativum*	23.3	0.0	Czech Republic, Central Bo	Lincoln

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
181	PI250448	*P. sativum*	39.1	0.0	Czech Republic, Central Bo	Libochovicky Rany
182	PI251051	*P. sativum*	37.1	0.3	Former Serbia and Montenegro	Col. No. 17236
183	PI253968	*P. sativum*	41.4	0.0	Afghanistan	Col. No. K1722
184	PI257244	*P. sativum*	22.2	0.6	China	Tung Haun
185	PI257592	*P. sativum*	33.4	0.6	Ethiopia, Shewa	G 9173
186	PI261622	*P. sativum*	27.3	2.1	Spain	Col. No. D-43
187	PI261623	*P. sativum*	36.6	2.1	Spain	Guisante Pirabesque
188	PI261624	*P. sativum*	20.7	0.3	Spain	Col. No. D-45
189	PI261631	*P. sativum*	15.4	0.0	Spain	No. D-47
190	PI261633	*P. sativum*	21.6	1.9	Spain	Telephone
191	PI261634	*P. sativum*	26.5	1.0	Spain	No. D-50
192	PI261635	*P. sativum*	27.3	1.3	Spain	No. D-87
193	PI261636	*P. sativum*	17.7	0.0	Spain	No. D-88
194	PI261671	*P. sativum*	23.1	0.0	Netherlands	Degrace
195	PI261677	*P. sativum*	34.3	1.0	Netherlands	Col. No. D-236
196	PI263014	*P. sativum*	31.0	0.6	Netherlands	Slier of Krombek
197	PI263027	*P. sativum*	17.6	2.8	France	Mongetout Corne De Belier
198	PI263028	*P. sativum*	22.5	0.6	France	Gullivert
199	PI263029	*P. sativum*	20.6	0.0	France	Nain Tres Hatif D'Armonay
200	PI263030	*P. sativum*	20.8	0.0	France	Pois Du Chemen Long
201	PI263031	*P. sativum*	16.3	1.5	France	Mangetout Carouby
202	PI263032	*P. sativum*	26.5	1.5	France	Pois Mangetout Fondant De Saint Diserat
203	PI263144	*P. sativum*	31.2	0.9	France	Col. No. D-282
204	PI263871	*P. sativum*	23.2	0.4	Greece	G 10331
205	PI266070	*P. sativum*	25.0	0.3	Sweden, Malmohus	Line No. 930
206	PI268480	*P. sativum* var. *arvense*	35.2	0.2	Afghanistan	Col. No. 317
207	PI269543	*P. sativum*	22.2	0.5	Pakistan	Col. No. 431
208	PI269544	*P. sativum*	13.0	0.1	Pakistan	Col. No. 854
209	PI269761	*P. sativum* var. *arvense*	35.6	0.1	Czech Republic	Aa135
210	PI269762	*P. sativum* spp. *sativum*	44.8	0.0	United Kingdom, England	Aa38

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
211	PI269770	*P. sativum*	30.4	0.5	United Kingdom, England	CN 31212
212	PI269777	*P. sativum*	26.6	0.0	United Kingdom, England	Aa87
213	PI269778	*P. sativum*	20.4	0.4	United Kingdom, England	Aa88
214	PI269782	*P. sativum*	21.9	1.6	United Kingdom, England	Aa92
215	PI269791	*P. sativum*	16.5	0.4	United Kingdom, England	Brochette
216	PI269793	*P. sativum*	33.1	0.5	United Kingdom, England	Telephone
217	PI269796	*P. sativum*	31.4	0.8	United Kingdom, England	Aa106
218	PI269797	*P. sativum*	23.4	2.5	United Kingdom, England	Aa107
219	PI269798	*P. sativum*	17.9	0.9	United Kingdom, England	Aa108
220	PI269802	*P. sativum*	21.6	2.6	United Kingdom, England	Aa112
221	PI269804	*P. sativum*	30.2	0.8	United Kingdom, England	Aa117
222	PI269810	*P. sativum*	35.7	0.0	United Kingdom, England	Aa125
223	PI269812	*P. sativum*	28.0	0.1	United Kingdom, England	Aa128
224	PI269816	*P. sativum*	25.7	0.3	United Kingdom, England	Aa132
225	PI269818	*P. sativum*	31.2	0.0	United Kingdom, England	Aa134
226	PI269821	*P. sativum*	20.0	0.0	United Kingdom, England	William Massey, Line 3
227	PI269822	*P. sativum*	21.4	0.0	United Kingdom, England	Perfection
228	PI269825	*P. sativum*	32.2	1.3	United Kingdom, England	Aa175
229	PI270536	*P. sativum*	29.0	0.6	Denmark	G 16703
230	PI271033	*P. sativum* spp. *abyssinicum*	36.7	0.0	Sweden, Malmohus	Abyssinicum
231	PI271035	*P. sativum*	24.6	1.8	Sweden, Malmohus	Hindukusch

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
232	PI271037	*P. sativum*	34.5	0.0	Sweden, Malmohus	Peluschke
233	PI271038	*P. sativum*	17.4	2.3	Nepal	Granart
234	PI271115	*P. sativum*	28.7	0.3	China	Ungar
235	PI271116	*P. sativum*	29.8	0.3	China	Granerbse
236	PI271118	*P. sativum*	23.4	0.8	China	Tibetanicum
237	PI271119	*P. sativum*	21.8	0.0	China	Puschki
238	PI271121	*P. sativum*	29.4	0.0	Germany	G 3586
239	PI271510	*P. sativum*	19.4	0.1	India	1293
240	PI271511	*P. sativum*	24.8	2.1	India	1295
241	PI272148	*P. sativum*	32.5	0.0	Finland	Navale Artturi
242	PI272152	*P. sativum*	28.8	1.3	Greece	Hiemale Landsorte
243	PI272166	*P. sativum*	28.7	0.3	Greece	Griseo Coloratum
244	PI272171	*P. sativum*	27.8	0.5	Germany	Zeylanicum, Selectas Peluschke
245	PI272175	*P. sativum*	27.8	0.0	Germany	Zeylanicum
246	PI272184	*P. sativum*	24.9	0.1	Greece	Arvense
247	PI272194	*P. sativum*	21.4	1.4	Germany	Concolon
248	PI272195	*P. sativum*	24.2	0.0	Germany	Concolon
249	PI272204	*P. sativum*	31.2	0.0	Germany	Hodinger Futtererbse
250	PI272207	*P. sativum*	25.1	0.1	Greece	Centrale-Sibiricum
251	PI272215	*P. sativum*	29.6	1.2	Germany	Lucienhofer Wintererbse
252	PI272216	*P. sativum*	29.4	0.3	Bulgaria	Winter-Futtererbse
253	PI272217	*P. sativum*	32.8	0.4	Germany	Wurtembergische Wi-Erbse
254	PI272218	*P. sativum*	29.2	0.0	Poland	Population
255	PI273207	*P. sativum*	34.8	1.3	Bulgaria, Sofia	9006/60
256	PI273209	*P. sativum* spp. *elatius*	30.1	0.0	Russian Federation, Lening	9009/60
257	PI273605	*P. sativum*	19.5	0.0	Ecuador	G 11058
258	PI274307	*P. sativum*	25.9	0.3	Pakistan	809
259	PI274308	*P. sativum*	23.3	0.3	Pakistan	815
260	PI274584	*P. sativum*	26.6	0.0	Norway	G 11173
261	PI275821	*P. sativum*	23.2	0.2	Sweden	G11250
262	PI275822	*P. sativum*	19.4	0.0	Sweden	Witham Wonder
263	PI275825	*P. sativum*	21.3	0.0	Sweden	G 11254
264	PI275826	*P. sativum*	23.3	0.3	Sweden	G 11255
265	PI277852	*P. sativum*	28.6	0.0	Ethiopia	No. 8090
266	PI279823	*P. sativum*	30.0	0.8	Germany	Edelperle
267	PI279824	*P. sativum*	20.8	0.4	Germany	Foli No. 1

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
268	PI279825	*P. sativum*	21.9	0.0	Germany	Foli No. 2
269	PI279827	*P. sativum*	35.9	0.0	Germany	Mansholt's Pluk
270	PI280236	*P. sativum*	24.7	0.0	Ethiopia	No. 7692
271	PI280252	*P. sativum*	26.9	0.1	Ethiopia	No. 8120
272	PI280603	*P. sativum*	36.5	0.0	Israel	Palestinicum
273	PI280606	*P. sativum*	28.9	0.3	Former Soviet Union	Amplissimo Local
274	PI280608	*P. sativum*	27.1	0.4	Afghanistan	Amplissimo Local
275	PI280609	*P. sativum*	29.3	0.0	Former Soviet Union	Amplissimo Local
276	PI280611	*P. sativum*	15.3	0.0	Ukraine	Amplissimo Victoria Ukrainskaya
277	PI280613	*P. sativum*	23.4	0.0	Former Soviet Union	Amplissimo Borec
278	PI280614	*P. sativum*	23.6	0.3	Former Soviet Union	Amplissimo Neistoscimyj
279	PI280616	*P. sativum*	30.7	0.0	Former Soviet Union	Amplissimo Zazerskij
280	PI280617	*P. sativum*	22.5	1.8	Estonia	Amplissimo Hamisepp
281	PI280619	*P. sativum*	18.1	0.4	Former Soviet Union	Amplissimo Tygevskij Kirju
282	PI280624	*P. sativum*	27.5	0.1	Former Soviet Union	Amplissimo Pozdnespelyj Mozgovoj
283	PI280626	*P. sativum*	17.2	0.2	Former Soviet Union	Amplissimo Yjuskij
284	PI285708	*P. sativum*	27.4	0.3	Poland, Warszawa	Czwartkowy Polny
285	PI285710	*P. sativum*	26.0	0.0	Poland, Warszawa	Kujawski Pozny
286	PI285715	*P. sativum*	22.6	0.8	Poland, Warszawa	Zolty Olbrazymi
287	PI285717	*P. sativum*	23.2	0.0	Poland, Warszawa	Nieznanicka Ciemna
288	PI285718	*P. sativum*	35.1	0.0	Poland, Warszawa	Pomorska
289	PI285719	*P. sativum*	22.6	0.3	Poland, Warszawa	Przebedowska Oliwkowa
290	PI285720	*P. sativum*	18.9	0.3	Poland, Warszawa	Pulawska Slodska No. 2
291	PI285722	*P. sativum*	16.7	0.1	Poland, Warszawa	Konserwowy I Har
292	PI285724	*P. sativum*	24.9	1.3	Poland, Warszawa	Lincoln-Freege
293	PI285727	*P. sativum*	37.2	0.3	Poland, Warszawa	Majowy
294	PI285730	*P. sativum*	32.1	0.1	Poland, Warszawa	Cud Ameryki
295	PI285737	*P. sativum*	30.3	0.4	Poland, Warszawa	Foli

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
296	PI285739	*P. sativum*	16.9	0.0	Poland, Warszawa	June Wonder
297	PI285740	*P. sativum*	31.4	1.2	Poland, Warszawa	Juvel
298	PI285747	*P. sativum*	31.9	0.2	Poland, Warszawa	Konservenkonigin
299	PI286430	*P. sativum*	26.4	0.0	Nepal	G 12600
300	PI286431	*P. sativum*	31.3	1.7	Nepal	Matar
301	PI286607	*P. sativum*	21.4	0.0	Thailand	G 12661
302	PI288024	*P. sativum*	31.8	0.0	France	Primcovert
303	PI288025	*P. sativum*	23.9	0.0	France	Quiki
304	PI288026	*P. sativum*	21.3	0.0	France	Ridesvert
305	PI288263	*P. sativum*	29.1	0.0	Germany	Mark-Erbsen
306	PI293426	*P. sativum*	26.6	0.2	Bulgaria, Sofia	No. 30
307	PI297081	*P. sativum*	25.2	0.6	Poland, Krakow	Szesciotygodniowy
308	PI299023	*P. sativum*	31.7	0.0	Greece	M 10
309	PI299024	*P. sativum*	24.0	0.0	Greece	M 129
310	PI306590	*P. sativum*	30.5	0.7	Hungary	Kreta 40
311	PI306591	*P. sativum*	21.8	0.0	Hungary	Pjeljuska Passzkaja
312	PI307666	*P. sativum*	29.1	2.0	Costa Rica	Verja
313	PI308796	*P. sativum*	16.8	0.9	India	Sylvia
314	PI311112	*P. sativum*	28.2	0.0	Guatemala	Col. No. 21386
315	PI314794	*P. sativum*	19.2	1.8	Australia	CPI 12559
316	PI314795	*P. sativum*	22.1	0.0	Australia	CPI 12561
317	PI314797	*P. sativum*	37.5	1.5	Australia	CPI 15248
318	PI314798	*P. sativum*	30.5	0.0	Australia	CPI 15456
319	PI314800	*P. sativum*	25.7	0.3	Australia	CPI 15742
320	PI314802	*P. sativum*	35.6	0.3	Australia	CPI 16428
321	PI314803	*P. sativum*	39.1	0.3	Australia	CPI 22002
322	PI316586	*P. sativum*	25.3	1.0	Australia, South Australia	White Brunswick
323	PI319374	*P. sativum*	20.7	2.6	Mexico	Col. No. 22074
324	PI320972	*P. sativum*	18.6	0.5	Hungary	Iregi Sarga IP. 1
325	PI324693	*P. sativum*	25.1	0.8	Hungary	Abesinijas
326	PI324695	*P. sativum*	32.9	0.0	Hungary	Arakass
327	PI324697	*P. sativum*	28.0	0.1	Hungary	Gris D’Hiver
328	PI324699	*P. sativum*	30.3	0.0	Hungary	Munchener-Banater-Wintererbse
329	PI324700	*P. sativum*	35.8	0.0	Hungary	Munchener Weissbluhende Wintererbse
330	PI324702	*P. sativum*	51.6	0.0	Hungary	Unra/972/

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
331	PI324703	*P. sativum*	44.1	0.0	Hungary	Wunterbergische-Wintererbse Population
332	PI324705	*P. sativum*	30.6	0.0	France	No. 830
333	PI324706	*P. sativum*	32.9	0.8	Romania	No. 833
334	PI331413	*P. sativum*	32.9	1.0	Ethiopia	Col. R-44
335	PI331414	*P. sativum*	48.4	0.0	Ethiopia	Col. 795-B
336	PI340128	*P. sativum*	43.7	0.0	Turkey	1-301
337	PI340130	*P. sativum*	32.2	0.0	Turkey	1-305
338	PI341889	*P. sativum*	30.9	1.0	Netherlands	Elwy
339	PI343077	*P. sativum*	17.5	0.8	Iran	G 19283
340	PI343271	*P. sativum*	39.3	0.3	United States, Idaho	G 18282
341	PI343292	*P. sativum*	39.9	0.0	United States, Idaho	G 18305
342	PI343321	*P. sativum*	21.2	0.0	United States, Idaho	G 18451
343	PI343331	*P. sativum*	36.4	2.0	United States, Idaho	G 18461
344	PI343338	*P. sativum*	22.2	0.1	United States, Idaho	G 18662
345	PI343824	*P. sativum*	39.1	1.0	Uganda	Col. 6946
346	PI343958	*P. sativum*	33.2	2.2	Turkey	Biselia
347	PI343968	*P. sativum*	33.9	2.5	Turkey	Beseliya (Local name)
348	PI343972	*P. sativum*	43.3	0.0	Turkey	22630
349	PI343973	*P. sativum*	57.5	0.0	Turkey	22631
350	PI343975	*P. sativum*	47.8	0.0	Turkey	22633
351	PI343976	*P. sativum*	54.2	0.5	Turkey	22716
352	PI343977	*P. sativum*	35.4	1.3	Turkey	22717
353	PI343979	*P. sativum*	54.2	0.0	Turkey	22725
354	PI343985	*P. sativum* spp. *sativum*	49.7	0.0	Turkey	22713
355	PI343987	*P. sativum* spp. *sativum*	39.4	0.5	Turkey	22718
356	PI343988	*P. sativum* spp. *sativum*	39.8	0.3	Turkey	22719
357	PI343990	*P. sativum* spp. *sativum*	23.5	0.0	Turkey	22722
358	PI344001	*P. sativum* spp. *elatius*	46.1	0.0	Turkey	22701
359	PI344002	*P. sativum* spp. *elatius*	48.9	0.0	Turkey	22702
360	PI344003	*P. sativum* spp. *elatius*	46.2	0.0	Turkey	22703

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
361	PI344005	*P. sativum* spp. *elatius*	47.5	0.0	Greece	22611
362	PI344006	*P. sativum* spp. *elatius*	45.2	0.0	Greece	22618
363	PI344007	*P. sativum* spp. *elatius*	40.8	0.0	Greece	22621
364	PI344009	*P. sativum* spp. *elatius*	57.8	0.0	Greece	22729
365	PI344010	*P. sativum* spp. *elatius*	45.3	0.0	Greece	22732
366	PI344011	*P. sativum* spp. *elatius*	43.1	0.0	Greece	22733
367	PI344012	*P. sativum* spp. *elatius*	50.5	0.0	Greece	22734
368	PI344013	*P. sativum* spp. *elatius*	35.0	0.3	Greece	22735
369	PI344537	*P. sativum* spp. *elatius*	49.9	0.0	Italy, Sicily	1
370	PI344538	*P. sativum* spp. *elatius*	59.0	0.0	Italy, Sicily	2
371	PI344539	*P. sativum* spp. *elatius*	33.2	0.0	Italy, Sicily	3
372	PI347281	*P. sativum*	33.7	2.3	India, Uttar Pradesh	PLP 10
373	PI347295	*P. sativum*	28.1	1.8	India, Uttar Pradesh	PLP 26
374	PI347457	*P. sativum*	28.3	2.0	India, Uttar Pradesh	PLP 264
375	PI347461	*P. sativum*	36.6	1.5	India, Uttar Pradesh	PLP 295
376	PI347477	*P. sativum*	40.3	0.3	India, Uttar Pradesh	PLP 503
377	PI347490	*P. sativum*	29.1	0.4	India, Uttar Pradesh	PLP 82
378	PI347495	*P. sativum*	27.5	0.6	India, Uttar Pradesh	PLP 97
379	PI347496	*P. sativum*	32.2	1.7	India, Uttar Pradesh	PLP 105A
380	PI347501	*P. sativum*	30.1	0.5	India, Punjab	PLP 132
381	PI355905	*P. sativum*	22.5	0.4	Japan	Kairyo Aotenashi
382	PI355906	*P. sativum*	27.0	0.3	Japan	Komidori
383	PI356973	*P. sativum*	27.3	0.5	India, Uttar Pradesh	PLP 35
384	PI356974	*P. sativum*	15.8	1.9	India, Punjab	PLP120
385	PI356980	*P. sativum*	34.5	0.8	India, Uttar Pradesh	PLP 16A
386	PI356984	*P. sativum*	36.1	0.4	India, Uttar Pradesh	PLP 70
387	PI356986	*P. sativum*	41.1	0.0	India, Punjab	PLP 174
388	PI356991	*P. sativum*	34.5	1.6	India, Haryana	PLP 196
389	PI356992	*P. sativum*	32.7	2.1	India, Haryana	PLP 197A
390	PI357020	*P. sativum*	27.6	1.8	India, Uttar Pradesh	PLP 349A

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
391	PI357048	*P. sativum*	34.4	0.8	India, Uttar Pradesh	PLP 314
392	PI357289	*P. sativum*	12.2	2.7	Former Serbia and Montenegro	Ran
393	PI357290	*P. sativum*	22.6	2.0	Former Serbia and Montenegro	Brzak
394	PI357292	*P. sativum*	12.6	2.0	Former Serbia and Montenegro	Kiflica
395	PI357293	*P. sativum*	22.6	1.5	Former Serbia and Montenegro	Debarski
396	PI357295	*P. sativum*	25.4	1.4	Former Serbia and Montenegro	Ran Sekerec
397	PI358300	*P. sativum*	44.8	0.0	Ethiopia	27b
398	PI358607	*P. sativum* spp. *abyssinicum*	39.4	0.0	Ethiopia	22770A
399	PI358608	*P. sativum* spp. *abyssinicum*	31.4	1.9	Ethiopia	22770B
400	PI358609	*P. sativum* spp. *abyssinicum*	34.5	0.0	Ethiopia	Wat
401	PI358610	*P. sativum* spp. *abyssinicum*	35.9	0.0	Ethiopia	22859
402	PI358611	*P. sativum* spp. *abyssinicum*	37.2	0.5	Ethiopia	22861
403	PI358612	*P. sativum* spp. *abyssinicum*	41.6	0.0	Ethiopia	22864
404	PI358613	*P. sativum* spp. *abyssinicum*	40.9	0.0	Ethiopia	22867
405	PI358614	*P. sativum* spp. *abyssinicum*	37.4	0.0	Ethiopia	22869
406	PI358615	*P. sativum* spp. *abyssinicum*	32.7	0.8	Ethiopia	22870
407	PI358616	*P. sativum* spp. *abyssinicum*	35.0	0.0	Ethiopia	22872

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
408	PI358617	*P. sativum* spp. *abyssinicum*	32.2	0.5	Ethiopia	22873
409	PI358620	*P. sativum*	49.2	0.0	Ethiopia	22758
410	PI358623	*P. sativum*	37.5	1.8	Ethiopia	22764
411	PI358628	*P. sativum*	40.4	0.8	Ethiopia	22771
412	PI358633	*P. sativum*	39.3	0.4	Ethiopia	22778
413	PI358640	*P. sativum*	43.7	0.4	Ethiopia	22791
414	PI358666	*P. sativum*	35.5	0.3	Ethiopia	22829
415	PI358700	*P. sativum*	32.8	0.4	Ethiopia	22892
416	PI365419	*P. sativum*	24.2	0.3	Canada, Manitoba	BR 1-49-9
417	PI365420	*P. sativum*	17.7	0.5	Canada, Manitoba	BR 2-29-1
418	PI371796	*P. sativum*	25.2	0.9	New Zealand	G 22442
419	PI378157	*P. sativum*	33.6	0.7	Malaysia	Red Flower No. 1
420	PI378158	*P. sativum*	25.1	1.8	Malaysia	White Flower No. 2
421	PI378159	*P. sativum*	32.8	1.1	Malaysia	White Flower No. 9
422	PI378160	*P. sativum*	36.3	2.3	Malaysia	G 22830
423	PI381334	*P. sativum*	37.5	0.1		Imposant Brown
424	PI390781	*P. sativum*	32.4	1.1	Peru	Ayacucho 24
425	PI390795	*P. sativum*	29.3	0.8	Peru	Junin 7
426	PI391630	*P. sativum*	34.0	2.8	China, Guangdong	Yi (“One” Pea)
427	PI392019	*P. sativum*	17.6	0.8	Former Soviet Union	Line 340/11
428	PI393488	*P. sativum*	46.0	0.0	Czech Republic, Central Bo	Klatovska Ozima
429	PI393489	*P. sativum*	30.1	0.0	Czech Republic, Central Bo	Munchen Weisbluhende
430	PI393490	*P. sativum*	16.5	1.4	Czech Republic, Central Bo	Spath’s Weinhenstephaner
431	PI404220	*P. sativum*	24.8	0.0	Former Soviet Union	Krasnoufimskij 70
432	PI404225	*P. sativum*	40.5	0.0	Former Soviet Union	Pinskij Mestnyj
433	PI409031	*P. sativum*	23.2	0.3	Germany	Hohenheimer Rosabluehende
434	PI411141	*P. sativum*	12.9	0.8	New Zealand, South Island	Pania
435	PI411142	*P. sativum*	17.7	0.5	New Zealand, South Island	Patea
436	PI411143	*P. sativum*	34.8	0.2	New Zealand, South Island	Piri

No	PI	Taxonomy	Mean Lesion Score	Mean Nodal Score	Country of Origin	Plant ID
437	PI411144	*P. sativum*	15.6	0.0	New Zealand, South Island	Puke
438	PI413678	*P. sativum*	26.9	0.7	Hungary	Zloty Olbrzymi
439	PI413679	*P. sativum*	20.5	0.3	Hungary	Marigreen
440	PI413683	*P. sativum*	18.6	0.0	Hungary	Lancet
441	PI413684	*P. sativum*	16.5	0.0	Hungary	Vitalis
442	PI413685	*P. sativum*	14.8	0.3	Hungary	NZ 51
443	PI413686	*P. sativum*	21.9	2.1	Hungary	Felicitas
444	PI413688	*P. sativum*	25.5	1.4	Hungary	Ujmajori Korai Victoria
445	PI413690	*P. sativum*	18.2	1.8	Hungary	Budai Csemege
446	PI413695	*P. sativum*	13.9	0.8	Hungary	Konzerv Gyongye
447	PI413697	*P. sativum*	24.2	2.0	Hungary	Mansfelder Grune
448	PI413698	*P. sativum*	28.3	0.9	Hungary	Grune Perle
449	PI413703	*P. sativum*	21.4	0.3	Hungary	I.P. 2
450	PI419217	*P. sativum*	39.6	1.0	Hong Kong	G 25353
451	PI429839	*P. sativum*	48.7	0.8	Afghanistan	Musus
452	PI429843	*P. sativum*	39.0	0.1	Former Soviet Union	Torsdag
453	PI429845	*P. sativum*	28.5	0.4	Former Soviet Union	Kombajnovyj 5
454	PI429849	*P. sativum*	44.8	0.5	Former Soviet Union	Uzbekskij
455	PI429853	*P. sativum*	37.1	2.5	Japan	Shirobana-Kinusaya
456	PI429856	*P. sativum*	17.6	0.5	Israel	Borec
457	PI433560	*P. sativum*	35.4	0.0	Israel	G 25945
458	PI476409	*P. sativum*	32.9	1.4	Latvia	Rota
459	PI476410	*P. sativum*	28.3	0.6	Ukraine	Mironovskij 186
460	PI476413	*P. sativum*	39.2	0.3	Russian Federation	Ul'ianovskij-72
461	PI477371	*P. sativum*	22.1	2.6	Denmark	Rosakrone
462	PI486131	*P. sativum*	55.7	0.5	Ecuador	E8454-A-F
463	PI494077	*P. sativum*	26.6	0.5	Chile	G 27915
464	PI499982	*P. sativum*	40.5	0.0	China	G 28097
465	PI505059	*P. sativum* spp. *elatius*	65.6	0.0	Sudan	Ilca 5076
466	PI505062	*P. sativum* spp. *sativum*	45.3	0.6	Greece	Ilca 5005
467	PI505080	*P. sativum*	55.9	0.8		Ilca 5039
468	PI505092	*P. sativum* spp. *sativum*	44.6	0.0	Cyprus	Ilca 5052

| No | PI | Taxonomy | Mean | Mean | Country of Origin | Plant ID |

			Lesion Score	Nodal Score		
469	PI505108	*P. sativum* spp. *sativum*	52.0	0.0	Greece	Ilca 5072
470	PI505111	*P. sativum* spp. *sativum*	51.8	0.0	Syria	Ilca 5075
471	PI505112	*P. sativum* spp. *sativum*	46.7	0.0	Greece	Ilca 5077
472	PI505120	*P. sativum* spp. *sativum*	37.6	0.0	Syria	Ilca 5085
473	PI505122	*P. sativum* spp. *sativum*	52.2	0.3	Albania	Ilca 5089
474	PI505127	*P. sativum* spp. *sativum*	46.1	0.0	Albania	Ilca 5094
475	PI505144	*P. sativum* spp. *sativum*	30.9	1.4	Spain	Ilca 5115
476	PI513252	*P. sativum* spp. *elatius*	37.4	0.0	Pakistan	Massar
477	PI533687	*P. sativum*	34.2	0.0	Spain	Chicharos
478	PI560055	*P. sativum* spp. *elatius*	33.7	0.1	Russian Federation	L. 89
479	PI560056	*P. sativum* spp. *elatius*	43.0	0.1	Russian Federation	L. 90
480	PI560057	*P. sativum* var. *pumilio*	45.7	0.0	Portugal	L. 92
481	PI560060	*P. sativum* spp. *elatius*	40.2	0.2	Israel	L. 106
482	PI560068	*P. sativum* var. *pumilio*	59.0	0.0	Israel, Jerusalem	L. 99
483	PI560072	*P. sativum* var. *pumilio*	41.5	0.0	Israel	L. 103
484	PI560969	*P. sativum* spp. *elatius*	56.4	0.0	Turkey	190785-0105