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# GENETIC CHARACTERIZATION AND MAPPING OF LATE BLIGHT RESISTANCE GENES IN THE WILD TOMATO ACCESSIONS PI 163245 AND PI 224710

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**ABSTRACT**

Late blight (LB), caused by the oomycete *Phytophthora infestans* (Mont.) de Bary is one of the most destructive diseases of tomato and potato worldwide. Development of fungicide resistant and more aggressive *P. infestans* clonal lineages has emphasized the importance of discovering and incorporating new genetic resistance in tomato cultivars. Although the cultivated tomato, *Solanum lycopersicum* L., contains limited genetic diversity, several related wild species of tomato are suitable for identification of new desirable traits. Previously, 67 *S. pimpinellifolium* accessions were screened for LB resistance in field, greenhouse and detached leaflet trials and 12 accessions with strong resistance to LB were identified. In this dissertation, two resistant accessions, PI 163245 and PI 224710, were selected for further genetic characterization. PI 163245 and PI 224710 were each hybridized with a LB susceptible tomato breeding line, Fla. 8059, and $F_1$ progeny were self-fertilized to develop $F_2$ populations. Large $F_2$ populations were grown and screened for LB resistance under greenhouse conditions, and the most resistant and most susceptible individuals in each $F_2$ population were retained for conducting heritability studies as well as identifying and mapping of resistance loci.

To characterize the genetic basis of resistance in the two accessions, estimates of heritability ($h^2$) were obtained based on $F_2$:$F_3$ parent-offspring (P:O) correlation analyses. An additional estimate of $h^2$ was obtained based on $F_3$:$F_4$ generations when using the accession PI 163245. Estimates of $h^2$ were moderately-high for both PI 163245 ($h^2_{F2:F3}$ = 0.78, $h^2_{F3:F4}$ = 0.94) and PI 224710 ($h^2_{F2:F3}$ = 0.87). The heritable nature of the resistance suggested that PI 163245 and PI 224710 were potentially viable for breeding LB resistance in tomato, and that mapping of LB resistance loci was warranted.

To discover SNP markers for genetic mapping studies, reduced representation libraries (RRLs) for each of PI 163245, PI 224710 and Fla. 8059 were constructed and sequenced. Comparisons of accessions PI 163245 and PI 224710 with breeding line Fla. 8059 resulted in the identification of 33,385 and 20,894 single nucleotide polymorphisms (SNPs), respectively. The most resistant (n = 39) and susceptible (n = 35) $F_2$ individuals in the PI 163245 mapping population were genotyped with 233 SNP markers, which were distributed throughout the genome. This selective genotyping approach identified four genomic intervals (quantitative trait loci, QTLs) on chromosomes 2, 3, 10, and 11 associated with LB resistance. Similarly, the most resistant (n = 40) and susceptible (n = 40) $F_2$ individuals in the PI 224710 mapping population were genotyped with 144 SNPs, and LB resistance QTLs were identified on chromosomes 1, 2, 10, and 12. Resistance QTLs on chromosomes 2 and 10 co-localized with two previously identified LB resistance genes, *qPh2.1* and *Ph-2* respectively, however fine mapping and cloning is necessary to determine how these QTLs correspond to previously identified LB resistance genes. The remaining resistance QTLs did not appear to correspond to known LB resistance genes or QTLs in tomato, and thus are likely unique to this study. Efforts to fine map these resistance QTLs and incorporate them into elite tomato breeding lines are currently in progress.

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# Chapter 1 Introduction

## Tomato

The cultivated tomato, *Solanum lycopersicum* L., is one of the most important crops worldwide. Tomato production of close to 164 million metric tons annually is valued at nearly $60 billion, ranking it first among vegetable crops and in the top ten food and agricultural commodities worldwide (FAOSTAT3.FAO.ORG). Tomatoes are also the second most commonly consumed vegetable crop, after only potatoes. Consequently, tomato products contribute substantially to the dietary intake of important nutrients per capita. In the United States, tomato is the number one dietary source of vitamins, minerals, and phenolic antioxidants (RICK 1980; NGUYEN AND SCHWARTZ 1999). Furthermore, tomatoes are the primary source of the antioxidant lycopene, which may reduce the risk of certain types of cancer (GIOVANNUCCI 1999).

*Solanum lycopersicum* is a member of the Solanaceae family, which consists of more than 90 genera and 3000-4000 species (KNAPP *et al.* 2004). Solanaceae contains many economically important species including tomato, potato, eggplant, pepper, tobacco, and petunia. While the geographic location of tomato domestication is unclear, isozyme evidence suggests Mexico as the most probable origin, while many wild relatives of tomato originated in Peru (RICK AND HOLLE 1990). However, available genetic evidence is inconclusive and domestication may have occurred independently in both regions (PERALTA AND SPOONER 2005). Following domestication, the tomato was brought to Europe and this germplasm likely composes a significant portion of the genetic background of modern cultivars. This major bottleneck considerably reduced the genetic diversity within the cultivated tomato (RICK AND FOBES 1975). Estimates suggest

as little as 5% of the total genetic variation within tomato species is available in the cultivated type (MILLER AND TANKSLEY 1990). However, several wild tomato species are suitable for trait discovery and have become an important genetic resource for improvement of horticultural and nutritional characteristics, biotic and abiotic stress tolerances, and disease resistance.

Wild tomato species are an invaluable resource for trait discovery and several *Solanum* species are easily hybridized with *S. lycopersicum*. While there is relatively little diversity within the cultivated germplasm, use of intercrossable wild tomato species has greatly expanded the genetic resources available for study. More than 80,000 cultivated and wild tomato accessions are obtainable from tomato seedbanks such as the Asian Vegetable Research and Development Center (AVRDC), United States Department of Agriculture (USDA), Plant Genetic Resources Unit at Geneva (PGRU), and the CM Rick Tomato Genetics Resource Center (TGRC) (BAUCHET AND CAUSSE 2012). Previously, nine species of tomato were recognized based on their ability to intercross and were categorized within either the Esculentum group or Peruvianum group (RICK *et al.* 1990). However, the number of species was later expanded to 13, encompassing regions from Ecuador to Bolivia and Chile, and including two species native to the Galapagos Islands (PERALTA *et al.* 2008). The 13 tomato species were assigned to four subgroups: Lycopersicon, Neolycopersicon, Eriopersicon, and Arcanum (PERALTA *et al.* 2008). The most closely related wild relatives to the cultivated tomato include *S. pimpinellifolium, S. galapagense,* and *S. cheesemanii,* all three of which belong to the Lycopersicon group (PERALTA *et al.* 2008). *S. pimpinellifolium* has been a particularly valuable resource, as it is the most closely related species to the cultivated

tomato. Consequently, *S. pimpinellifolium* has become one of the most commonly used wild tomato species in cultivar improvement for disease resistance, biotic and abiotic stress tolerances, and fruit quality.

**Tomato as a model organism**

In addition to its importance as a food crop, tomato serves as a model organism for both basic and applied research and is genetically one of the most thoroughly mapped flowering plants (RICK AND YODER 1988; FOOLAD 2007; SIM *et al.* 2012). Tomato is especially important as a model for fleshy fruit development and evolution (MEISSNER *et al.* 1997; CONSORTIUM 2012) and continues to be used as a model for the entire Solanaceae family. A number of factors contribute to the usefulness of tomato as a genetic model. Tomato species contain just 12 chromosomes (2n = 2x = 24) and are diploid, representing a relatively simple genetic system (RICK AND BUTLER 1956). The tomato genome is a modest size of approximately 900 Mbp (CONSORTIUM 2012) and contains fewer repetitive sequences than many other plant species (ZAMIR AND TANKSLEY 1988; CONSORTIUM 2012). Additionally, many morphological characteristics are easily detectable including fruit, flower and leaf morphology, growth habit, and fruit color which facilitated development of early genetic maps prior to widespread molecular marker availability (RICK AND YODER 1988). These genomic and morphological differences were expanded by utilizing intercrossable wild tomato species for map development. In addition to the large selection of naturally occurring and traditionally bred tomato accessions, large mutant populations consisting of thousands of $M_2$ families are available for study (MEISSNER *et al.* 1997; MENDA *et al.* 2004).

Further contributing to its ease of research, most tomato species are easily self-pollinated or hybridized, have a relatively short life cycle, and are day neutral (RICK AND YODER 1988). Additionally, the tomato is easily transformed via *Agrobacterium tumefaciens* allowing for rapid transfer of cloned genes (RICK AND YODER 1988). In fact, the first marketed genetically engineered food crop was the FLAVR SAVR tomato released by Calgene in 1994, for which shelf life was increased by suppression of the polygalacturonase enzyme (BREUNING AND LYONS 2000). As a result of these desirable characteristics, a wealth of molecular and morphological markers, ESTs, YAC and BAC libraries as well as many cytological, physical, and genetic maps are available (FOOLAD 2007; GUPTA *et al.* 2009).

**The tomato genome**

The complete tomato genome sequence of the processing tomato accession Heinz 1706 was released in 2012 and serves as a reference genome for the assembly of new genomic sequences and identification of genetic markers or genes of interest (CONSORTIUM 2012). The genome consists of approximately 35,000 protein-coding genes and more than 30,000 were supported by RNA sequencing (VAN DER HOEVEN *et al.* 2002; CONSORTIUM 2012). The cultivated tomato genome is highly similar to the draft genome of *S. pimpinellifolium,* displaying only a 0.6% nucleotide divergence, confirming the close relationship between the two species (CONSORTIUM 2012). Despite the high sequence similarities, 5.4 million SNPs were identified between the two genomes (CONSORTIUM 2012). Additionally, comparisons of more than 31,000 genes indicated close to 13,000 non-synonymous mutations (CONSORTIUM 2012). More recently, 360 tomato accessions, consisting of both cultivated and wild tomato accessions, were

sequenced and 11.6 million SNPs, 207,306 non-synonymous SNPs affecting 30,945 genes, and 1.3 million small InDels distributed throughout the genome were identified (LIN *et al.* 2014). Before the development of SNP marker technology, the close relationship between *S. lycopersicum* and *S. pimpinellifolium* could be problematic, often resulting in insufficient genetic markers for mapping. However, next generation sequencing (NGS) and new cost-effective SNP genotyping techniques have greatly alleviated this issue.

**Genetic mapping of tomato**

Although the costs of whole genome sequencing have fallen dramatically over the past several years, genetic mapping remains integral for discovering and implementing new genes in tomato breeding. Despite advances in *in silico* candidate gene prediction, traditional mapping studies are needed for delineating genomic regions associated with desired traits. Trait discovery, identification of desirable germplasm, and gene introgression are still largely based on traditional recombination studies. The majority of linkage maps developed for tomato have been based on interspecific crosses, particularly between the cultivated tomato and *S. pennellii*, *S. pimpinellifolium,* and *S. habrochaites,* in order to take advantage of more abundant genetic dissimilarities between different species (FOOLAD 2007).

The earliest linkage map in tomato was generated in 1968 and was based on 153 morphological and physiological markers (BUTLER 1968). This early linkage map was later used in assembling the first genetic map of tomato utilizing isozyme markers (TANKSLEY AND RICK 1980). The first genetic map in tomato based on genomic markers was released in 1986 and used a combination of 18 isozyme and 94 DNA markers, which

consisted primarily of cDNA clones (BERNATZKY AND TANKSLEY 1986). Just six years later, the first high density linkage map in tomato was released, containing more than 1,000 markers and based on an interspecific cross of *S. lycopersicum* and *S. pennellii* (TANKSLEY *et al.* 1992). The map was further expanded over the next ten years and by 2002 more than 2,500 molecular markers had been added (FULTON *et al.* 2002).

Cost effective SNP genotyping and large-scale SNP identification via NGS allowed construction of several high density molecular maps in tomato based on crosses between *S. lycopersicum* (LA0925) x *S. pennellii* (LA0716), *S. lycopersicum* (Moneymaker) x *S. pennellii* (LA0716), and Moneymaker x *S. pimpinellifolium* (LA0121). These mapping populations were genotyped with 3,503, 3,687, and 4,491 SNPs, respectively (SIM *et al.* 2012). As a result of diminishing genotyping costs and the ability to discover large numbers of SNP markers, these types of large scale mapping studies are becoming more feasible. Currently, more than 25 tomato linkage maps are available (FOOLAD 2007).

The most frequently used mapping populations in tomato are early filial (i.e. $F_2$) and early backcross (i.e. $BC_1$) populations due to their ease of development and high degrees of linkage disequilibrium (LD). However, experiments utilizing these types of populations face several limitations including low repeatability, evaluations based on individual plant performances (i.e. no replications), limited mapping resolution, reduced accuracy of QTL detection when using wide-crosses, and unstable genetic backgrounds. Furthermore, these populations are not immediately useful for breeders due to undesirable genetic backgrounds and high potential for linkage drag. In contrast, recombinant inbred lines (RILs), advanced backcross, backcross inbred lines (BILs), and

introgression lines (ILs), while more cost prohibitive and time consuming to develop, allow replication of experiments, increased mapping resolution, and have more stable genetic backgrounds (FOOLAD 2007). However, higher marker density is required in these advanced mapping populations due to lower LD and consequently they may be less desirable for preliminary QTL mapping.

Genetic mapping of tomato previously relied on molecular markers such as RFLPs, AFLPs, SSRs, CAPS, RGAs, ESTs, and COSs (FOOLAD 2007). These markers are becoming less frequently used due to factors such as low reproducibility, high labor requirements, and limited degrees of polymorphism. While traditional genomic markers may still be applicable for small-scale studies, the abundance and cost effectiveness of SNPs make them an ideal choice. The availability of a reference genome sequence and the innovations of relatively inexpensive high-throughput sequencing and genotyping techniques facilitate the ability to discover and genotype tremendous numbers of markers. Developing population-specific SNPs using NGS has become possible even for smaller research groups.

Creating a genetic map and identifying markers associated with desired horticultural traits, including improved fruit quality, abiotic stress tolerance, and disease resistance, is the first step in modern cultivar enhancement. Improving disease resistance is an area of great importance in the tomato breeding community. Worldwide, the cultivated tomato is susceptible to more than 200 diseases caused by fungi, bacteria, viruses, and nematodes depending on geographic location (LUKYANENKO 1991). One disease of growing importance on tomato is late blight (LB), caused by the pathogen *Phytophthora infestans.*

**Late blight**

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most devastating diseases of tomato and potato worldwide. In tomato, late blight accounts for as much as 7% yield losses annually in the United States and similar losses are incurred globally (HTTP://WWW.NASS.USDA.GOV/QUICK_STATS ; NOWICKI *et al.* 2012). In potato alone, annual costs associated with LB are valued at more than $6 billion worldwide (HAVERKORT *et al.* 2008; HAVERKORT *et al.* 2009).

Late blight can affect all above-ground portions of the plant as well as the potato tuber and symptoms of the disease include dark brown, purple, or black lesions. Late blight particularly thrives under cool and humid conditions. In especially high humidity, sporangia may appear on the abaxial (lower) side the leaf. Late blight is able to rapidly destroy susceptible potato and tomato tissue within a few days of infection and is notoriously difficult to manage. Several factors contribute to the effectiveness of *P. infestans* as a pathogen. Initial disease symptoms are difficult to detect and discovery is often too late for effective fungicide treatment. Additionally, each lesion may produce as many as 300,000 sporangia each day, allowing for rapid dispersal of the pathogen. *P. infestans* also has a short asexual life cycle often lasting fewer than five days (FRY AND GOODWIN 1997). Lastly, the *P. infestans* genome (discussed below) encourages effector evolution, facilitating breakdown of host resistance.

*Phytophthora infestans* is a heterothallic organism, requiring both A1 and A2 mating types for sexual reproduction. Until recently only the A1 mating type was commonly found outside of Mexico (FOOLAD *et al.* 2008). However, during the 1980s the A2 mating type was identified in Europe and the United States, potentially allowing for

more rapid development of new and more aggressive clonal lineages (HOHL AND ISELIN 1984; DEAHL *et al.* 1991). Despite the occurrence of both mating types within the United States, only two reported instances of sexual *P. infestans* populations have been reported (GAVINO *et al.* 2000; DANIES *et al.* 2014). However, the frequency of sexual populations in Europe appears to have increased (YUEN AND ANDERSSON 2013). Further increasing the difficulty of managing LB, is the development of phenylamide resistance in *P. infestans*, which is of great concern to tomato and potato growers since phenylamides are one of few effective systemic fungicides (GISI AND COHEN 1996; GOODWIN *et al.* 1996). The occurrence of fungicide resistant *P. infestans* lineages and rapid breakdown of host resistance makes LB an extremely difficult disease to manage effectively.

***Phytophthora infestans***

The origin of *P. infestans* is debated, with evidence supporting origination in either the South American Andes or the Toluca Valley of Central Mexico (AUSTIN BOURKE 1964; ANDRIVON 1996; GRÜNWALD AND FLIER 2005; GÓMEZ-ALPIZAR *et al.* 2007). Proponents of the Andean theory point out that *P. infestans* would likely have originated in the same center of origin as potato, tomato, and other Solanaceous species. Additionally, sequencing of nuclear and mitochondrial genes seems to support the Andean theory (GÓMEZ-ALPIZAR *et al.* 2007). However, DNA fingerprinting of several *P. infestans* populations identified higher levels of genetic diversity in Mexico compared to the Andes, suggesting LB may have originated in the Toluca Valley (TOOLEY *et al.* 1989; GOODWIN *et al.* 1994; PEREZ *et al.* 2001). Furthermore, Solanaceous hosts for LB are found outside of the Andes, contrary to one of the primary arguments supporting the Andean origin. Most recently, the Mexican origin was supported using microsatellite

markers to fingerprint nuclear genes from several populations of *P. infestans* across Mexico, the Andes, and other regions. Phylogenetic analysis suggested Mexican origin for all four nuclear genes tested (GOSS *et al.* 2014).

*Phytophthora infestans* was initially believed to be closely related to fungi due to common shared traits including heterotrophy and filamentous growth. However, as an oomycete, *P. infestans* is more closely related to algae and diatoms (LAMOUR *et al.* 2007). There are two known physiological races of *P. infestans* consisting of T-0 and T-1 (CONOVER AND WALTER 1953; GALLEGLY 1960). Race T-0 is no longer considered problematic and the more aggressive race T-1 is believed to compose most *P. infestans* lineages (FOOLAD *et al.* 2008). The A1 and A2 mating types are differentiated by mating hormones as opposed to morphological differences (JUDELSON 1997b). The sexual life cycle, which produces new and potentially more aggressive clonal lineages, begins with interaction of mycelia between the two mating types and the release of mating hormones, stimulating growth of the male antheridia and female oogonia (collectively the gametangia). While most mating types are bisexual, certain isolates preferentially produce antheridia or oogonia depending on their mating partner (GALINDO AND GALLEGLY 1960; JUDELSON 1997a). The gametangia of the two mating types generate haploid nuclei via meiosis, which fuse and eventually produce diploid oospores. These oospores are long-lived and highly durable, allowing for survival in harsh conditions and potentially serving as inoculum for future outbreaks of the disease (JUDELSON 1997b). In contrast, while less enduring than the sexually produced oospores, the asexual life cycle of *P. infestans* enables rapid population growth beginning when sporangia or zoospores arrive on host tissue. Sporangia produce germ tubes at higher temperatures (20-25 ºC) or

release biflagellate zoospores in cooler temperatures (10-15 ºC), that encyst. Hyphae extend within host tissue and haustoria are produced, forming a biotrophic feeding relationship. Since *P. infestans* is hemibiotrophic, the host cells eventually perish (FRY 2008; JUDELSON 1997b). Specialized hyphae called sporangiophores are ultimately produced, which produce and release new sporangia (JUDELSON 1997b; FRY 2008).

### *Phytophthora infestans* genome

The *P. infestans* genome is approximately 240 Mb and contains nearly 18,000 protein coding genes (HAAS *et al.* 2009). The majority of housekeeping genes are contained in clusters of high gene density with few repetitive regions. These high-density regions contain approximately 70% of all *P. infestans* genes, separated by just 603 bp on average. However, genes encoding apoplastic, RXLR, and CRN effector proteins occupy gene sparse regions of the genome and average 3.7 kb between genes (HAAS *et al.* 2009). Effector gene families in *P. infestans* are highly expanded compared to other sequenced *Phytophthora* species, likely facilitated by the repetitive nature of the genome, which encourages frequent non-allelic homologous recombination and tandem gene duplication events (HAAS *et al.* 2009). Overall, the *P. infestans* genome is highly repetitive. Repetitive sequences make up 74% and Gypsy elements constitute nearly one third of the genome. The dynamic nature of the *P. infestans* genome likely contributes to the high rates of gene gain and loss of function (HAAS *et al.* 2009).

### Late blight management

Management of late blight generally involves a combination of good cultural practices and frequent fungicide applications. Cultural practices associated with management of LB include crop rotation and fallow of potato fields, elimination of

sources of LB inoculum such as potato cull piles, and early detection and treatment of infected plants. Previously, fungicides containing phenylamides (e.g. mefenoxam) were effective against most *P. infestans* populations. However, by the 1980s phenylamide-resistant populations of *P. infestans* were identified frequently throughout the United States and Europe (GISI AND COHEN 1996; GOODWIN *et al.* 1996). Currently, a combination of protectant and translaminar fungicides are most commonly used in commercial tomato and potato operations. Effective protectant fungicides used in the management of tomato LB include chlorothalonil, copper hydroxide, and mancozeb, (STEVENSON 2008; NOWICKI *et al.* 2012). This list has expanded over the last several years and numerous other translaminar fungicides containing active ingredients such as cyazofamid, dimethomorph, and mandipropamid have been developed. While the number of fungicides has increased, there are fewer systemic fungicides available for growers, especially in conjunction with phenylamide-resistant *P. infestans* populations. Mefenoxam is still employed frequently against susceptible *P. infestans* lineages, and additional fungicides such as aliphatic nitrogen or morpholine based fungicides may be used (NOWICKI *et al.* 2012). Unfortunately, fungicides have financial and environmental costs that are not ideal for growers or consumers. Consequently, better utilization of tomato and potato cultivars containing LB resistance is necessary.

**Late blight resistance in potato**

*Phytophthora infestans* has tremendous evolutionary potential and a remarkable ability to overcome resistant cultivars (MCDONALD AND LINDE 2002; FRY 2008; HAAS *et al.* 2009). Consequently, it is necessary to identify and employ multiple LB resistance genes to achieve durable resistance. Plant defense against *P. infestans* is generally

characterized by hypersensitive response (HR) when *R* genes associated with LB resistance, termed *Rpi* genes, are activated by corresponding avirulence (Avr) factors (KAMOUN *et al.* 1999; VLEESHOUWERS *et al.* 2000). It is believed the majority of *Rpi* genes encode proteins belonging to the CC-NBS-LRR class, while all identified Avr proteins in *P. infestans* belong to the RXLR effector class (VLEESHOUWERS *et al.* 2011). More than 20 functional LB resistance genes have been cloned in potato and tomato, all of which encode CC-NBS-LRR proteins (JO *et al.* 2015). When examining LB resistance, it is important to discuss genetic resistance identified in both potato and tomato as significant synteny exists between the two species and the genomic positions of *R* genes often occur at corresponding loci (GRUBE *et al.* 2000). Late blight resistance genes have been identified and/or cloned in several potato species including *S. demissum* (BALLVORA *et al.* 2002; HUANG *et al.* 2005; LOKOSSOU *et al.* 2009; LI *et al.* 2011a), *S. bulbocastanum* (SONG *et al.* 2003; VAN DER VOSSEN *et al.* 2003; VOSSEN *et al.* 2005; LOKOSSOU *et al.* 2009), *S. stoloniferum* (VLEESHOUWERS *et al.* 2008), *S. venturii* (FOSTER *et al.* 2009; PEL *et al.* 2009), *S. chacoense* (VOSSEN *et al.* 2010), and *S. x edinense* (DE VETTEN *et al.* 2011). Late blight resistance has also been reported in *S. berthaultii* (EWING *et al.* 2000), *S. mochiquense*, *S. phureja*, and *S. pinnatisectum* (KUHL *et al.* 2001; SMILDE *et al.* 2005; SLIWKA *et al.* 2006).

*Rpi* genes identified in *S. demissum* were the first widely utilized LB resistance genes in potato breeding, and initially 11 race specific *R* genes (*R1-R11*) were identified (BLACK *et al.* 1953; MALCOLMSON 1969). However, all 11 of these *R* genes have been overcome by certain *P. infestans* isolates (BRADSHAW *et al.* 2006). *R1* was mapped to chromosome 5 (LEONARDS-SCHIPPERS *et al.* 1992) and later cloned (BALLVORA *et al.*

2002). While *R1* had been widely utilized, it currently has limited value since most clonal lineages can overcome the resistance gene (TROGNITZ AND TROGNITZ 2007). *R2* belongs to a diverse gene family located on chromosome 4 (LI *et al.* 1998). So far, 11 *R2* orthologs have been identified using a combination of effectoromics, map based cloning, and allele mining approaches across several *Solanum* species (VLEESHOUWERS *et al.* 2011). In addition to *S. demissum, R2* orthologs were identified in *S. bulbocastanum* (*Rpi-blb3*) (PARK *et al.* 2005a; LOKOSSOU *et al.* 2009), *S. edinense* (*R2-like, Rpi-edn1.1*) (PARK *et al.* 2005c; LOKOSSOU *et al.* 2009), *S. schenckii* (*Rpi-snk1.1, Rpi-snk1.2*) (PARK *et al.* 2005b; LOKOSSOU *et al.* 2009; CHAMPOURET 2010), *S. bjertingii* (*Rpi-bjt1.1, Rpi-bjt1.2*, *Rpi-bjt1.3*), and *S. microdontum* (*Rpi-mcd1*) (CHAMPOURET 2010). The *R3* resistance gene was mapped to chromosome 11, and was found to consist of two tightly linked *R* genes termed *R3a* and *R3b* (HUANG *et al.* 2005). An *R3* variant, *Rpi-sto2* was discovered in *S. stoloniferum* (CHAMPOURET 2010). The remaining *R* genes from *S. demissum, R4* through *R11*, were also mapped to chromosome 11 and are either tightly linked to *R3* or are potential allelic variants (EL-KHARBOTLY *et al.* 1996; HUANG *et al.* 2005; BRADSHAW *et al.* 2006; VERZAUX 2010).

Three race non-specific LB resistance genes have also been identified and mapped in *S. bulbocastanum*. The *RB* gene, mapped to chromosome 8 (SONG *et al.* 2003), was later cloned and renamed *Rpi-blb1* (VAN DER VOSSEN *et al.* 2003). *Rpi-blb1* displays broad spectrum resistance against many *P. infestans* isolates, however it was recently reported that certain *P. infestans* isolates can overcome *Rpi-blb1* (CHAMPOURET *et al.* 2009). The second resistance gene, *Rpi-blb2* was mapped to chromosome 6 and is located in a cluster of genes encoding NBS-LRR class proteins (VAN DER VOSSEN *et al.*

2003). Two variants of *Rpi-blb2* were identified in *S. stoloniferum*: *Rpi-sto1* and *Rpi-pta1* (VLEESHOUWERS *et al.* 2008). The third resistance gene identified in *S. bulbocastanum*, *Rpi-blb3*, was mapped to chromosome 4 and is considered part of the *R2* family (PARK *et al.* 2005a). While all three of these resistance genes display LB resistance against a range of *P. infestans* isolates, they have not been widely employed in potato breeding due to direct incompatibility between *S. bulbocastanum* and *S. tuberosum*. Discovery of these genes in compatible wild potato species such as *S. stoloniferum* could allow for more widespread use of these *Rpi* genes.

A *Tm-2*$^2$ homolog discovered in *S. venturrii* also provides resistance to LB. The resistance gene consists of three alleles, *Rpi-vnt1*, *Rpi-vnt1.2* and *Rpi-vnt1.3*, and was mapped to chromosome 9 (FOSTER *et al.* 2009; PEL *et al.* 2009). Despite displaying resistance against several *P. infestans* isolates, it has not been widely utilized in breeding (PEL *et al.* 2009). Several other resistance genes have also been mapped to chromosome 9 in *S. mochiquense* (*Rpi-moc1*) (SMILDE *et al.* 2005), *S. phureja* (*Rpi-phu1*) (SLIWKA *et al.* 2006), *S. dulcamara* (*Rpi-dlc1*) (GOLAS *et al.* 2010), and *S. caripense* (TROGNITZ *et al.* 2004). Interestingly, the tomato LB resistance gene *Ph-3,* also co-localizes to this region (CHUNWONGSE *et al.* 2002).

Late blight resistance genes have additionally been mapped in *S. pinnatisectum*, *S. berthaultii*, and *S. paucissectum* accessions. *S. pinnatisectum* confers a dominant resistance gene, *Rpi1*, and is located on chromosome 7 (KUHL *et al.* 2001). In *S. berthaultii*, three *Rpi* genes, *Rpi-ber* (RAUSCHER *et al.* 2006), *Rpi-ber1*, and *Rpi-ber2* (PARK *et al.* 2009), were mapped to the long arm of chromosome 10 to a similar genomic region as the *Ph-2* tomato LB resistance gene. Major QTLs for LB resistance were also

identified in *S. paucissectum* on chromosomes 10, 11, and 12, although only a QTL on chromosome 11 was detected in all experiments (VILLAMON *et al.* 2005). Lastly, a preliminary mapping study identified two LB resistance QTLs in *S. chacoense* on chromosomes 9 and 10 (CHAKRABARTI *et al.* 2014). Considerable efforts have been made to identify and utilize new sources of LB resistance in potato as evidenced by the large number resistance genes studied across many wild potato species. Comparatively, it is only recently that similar efforts have been initiated to identify LB resistance in tomato and its wild relatives. As a result, relatively fewer LB resistance genes have been mapped in tomato.

**Late blight resistance in tomato**

Three major LB resistance genes have been widely utilized in tomato breeding, although several other QTLs have been reported. The first LB resistance gene identified was *Ph-1*, a dominant gene conferring resistance to the *P. infestans* race T-0. *Ph-1* was discovered in *S. pimpinellifolium* accessions West Virginia 19 and 731, and was mapped to chromosome 7 (BONDE AND MURPHY 1952; GALLEGLY AND MARVEL 1955; PEIRCE 1971). Several tomato cultivars were developed utilizing this resistance gene including Rockingham, Nova, and New Yorker. However, the predominant *P. infestans* race has switched from T-0 to T-1 and consequently *Ph-1* is no longer particularly valuable for breeding purposes (FOOLAD *et al.* 2008). The second LB resistance gene, *Ph-2*, was identified a few years later in the *S. pimpinellifolium* accession West Virginia 700 (GALLEGLY AND MARVEL 1955). *Ph-2* exhibits incomplete dominance and has been overcome by certain *P. infestans* isolates or under high disease pressures (GOODWIN *et al.* 1995; BLACK *et al.* 1996; FOOLAD *et al.* 2008). *Ph-2* is located distally on the long arm of

chromosome 10 and was mapped to an 8.4 cM interval (MOREAU *et al.* 1998). While *Ph-2* is not particularly effective independently, when combined with *Ph-3* a very high level of resistance is observed and recently cultivars have been released containing both resistance genes (GARDNER AND PANTHEE 2010b; GARDNER AND PANTHEE 2010a; PANTHEE AND GARDNER 2010). *Ph-3* is currently considered the strongest single resistance gene in tomato and confers resistance to a broad spectrum of *P. infestans* isolates. *Ph-3* was identified in the *S. pimpinellifolium* accession L3708 (a.k.a. LA1269 and PI 365957) and mapped to the long arm of chromosome 9 (CHUNWONGSE *et al.* 2002). Currently, *Ph-3* is the only tomato LB resistance gene fine mapped and cloned. Similarly to cloned potato LB resistance genes, *Ph-3* encodes a CC-NBS-LRR protein (ZHANG *et al.* 2013; ZHANG *et al.* 2014). Further examination of the source of *Ph-3*, L3708, suggested that additional factors contribute to better LB resistance in the parental accession compared to breeding lines and cultivars containing *Ph-3* alone (KIM AND MUTSCHLER 2005; LEE *et al.* 2006). A second genetic linkage map was developed for L3708 using Restriction site Associated DNA Sequencing (RAD-Seq) and a minor resistance QTL was found on chromosome 2 (CHEN *et al.* 2014). However, this QTL has not been utilized in breeding. A recent evaluation of 67 *S. pimpinellifolium* accessions for LB resistance identified 12 accessions containing resistance comparable to *Ph-2* + *Ph-3* combined (FOOLAD *et al.* 2014b). One of the most resistant accessions, PI 270443 was further characterized and two genomic regions were associated with LB resistance. This resistance, denoted as *Ph-5*, was mapped to the long arms of chromosomes 1 and 10 and was highly heritable (MERK AND FOOLAD 2012). Breeding efforts are in progress

incorporating this resistance into tomato breeding lines (MERK *et al.* 2012; MERK AND FOOLAD 2012).

In addition to the aforementioned LB resistance genes, several QTLs have been identified that have not successfully been used in tomato breeding. Race non-specific LB resistance was identified in the *S. habrochaites* accession LA 2099 and QTLs were mapped to all 12 tomato chromosomes (BROUWER *et al.* 2004). The strongest of these QTLs, *lb4*, *lb5b,* and *lb11b* were fine mapped on chromosomes 4, 5, and 11 respectively (BROUWER AND ST. CLAIR 2004). Unfortunately, these QTLs are associated with many undesirable traits including poor plant shape and canopy density, low yield, small fruit size, and late maturity (BROUWER AND ST. CLAIR 2004). Four regions associated with LB resistance in the *S. habrochaites* accession LA 1777 co-localized with QTLs previously identified in LA 2099 (LI *et al.* 2011b). However, one novel QTL on chromosome 4 (*Rlbq*4b) was reported (LI *et al.* 2011b). A third LB resistant *S. habrochaites* accession, BGH6902, was estimated to contain as many as 28 genes associated in LB resistance, although none of these genes were mapped (ABREU *et al.* 2008). Late blight resistance was reported in the *S. pennellii* accession LA 716, and mapped to chromosome 6 (SMART *et al.* 2007). However, this resistance gene might not be particularly useful since the authors suggest the resistance could be conditioned by indeterminate growth habit rather than by true host resistance. Resistance likely controlled by two loci was identified in the *S. pimpinellifolium* accession L3707, however the genomic locations for this resistance are unknown (IRZHANSKY AND COHEN 2006).

**Dissertation research background**

The growing impact of LB resistance in tomato prompted the study and identification of new sources of LB resistance as a major research focus in The Pennsylvania State University's tomato breeding program. Previously, screening of approximately 300 *S. pimpinellifolium* accessions for numerous traits identified 67 accessions with desirable horticultural characteristics, including good fruit quality, abiotic stress tolerance, and disease resistance (FOOLAD, unpublished data). Subsequently, these 67 accessions were evaluated for resistance against several *P. infestans* isolates from the US-13, US-14, and US-23 clonal lineages in field, greenhouse, and detached leaflet studies (FOOLAD *et al.* 2014a; FOOLAD *et al.* 2014b). From these 67 accessions, 12 were highly resistant to LB and consistent with control lines containing *Ph-2* and *Ph-3* combined (FOOLAD *et al.* 2014b). Two of these accessions, PI 163245 and PI 224710, are the focus of this study.

**Research Objectives**

The goals for this project were two-fold for both PI 163245 and PI 224710. First, to determine the breeding utility of these accessions, the heritability of LB resistance was examined in interspecific segregating populations derived from these accessions. Heritability explains the proportion of total variance in a population attributable to the genetic variance of a trait and can be quantified in either the broad sense ($H^2$), consisting of both additive and dominance variance, or the narrow sense ($h^2$), consisting of only the additive variance. Calculating $h^2$ allows predictions to be made regarding the responses of populations and families to artificial and natural selection. Additionally, the probability of detecting genes with large effects on the desired phenotype increases in

conjunction with $h^2$, estimating the potential efficiency of gene mapping studies (VISSCHER *et al.* 2008). For many crop species, heritability can be reliably determined by parent-offspring (P:O) correlation analysis (FALCONER AND MACKAY 1996). PI 163245 and PI 224710 were each hybridized with a LB susceptible tomato breeding line (Fla. 8059) and subsequent filial generations were developed. Using $F_2$:$F_3$ and $F_3$:$F_4$ P:O correlation analyses, $h^2$ of LB resistance conferred by PI 163245 was estimated and the realized heritability ($h^2_R$) was also measured by response to selection (Chapter 2). The $h^2$ of resistance conferred by PI 224170 was estimated using $F_2$:$F_3$ P:O correlation and the $h^2_R$ was calculated (Chapter 3).

The second goal of this research was to determine the genomic intervals associated with LB resistance conferred by PI 163245 and PI 224710. With this goal in mind, genetic mapping studies were conducted. In order to perform genetic mapping, a large number of polymorphic markers distributed throughout the genome were needed. SNPs are the ideal candidate for mapping studies due to their abundance and declining costs of large-scale genotyping. One approach to SNP discovery is sequencing and comparing reduced representation libraries (RRLs) across genotypes. Reduced representation libraries are constructed using one or more restriction enzymes to reduce genome complexity. This approach was first utilized using the human genome (ALTSHULER *et al.* 2000) and has since been applied in soybean (HYTEN *et al.* 2010), sorghum (NELSON *et al.* 2011), and flax (KUMAR *et al.* 2012). Combined with the increasing availability of reference genome sequences and the efficiency of new genotyping platforms such as Taqman® (HOLLAND *et al.* 1991), GoldenGate® (FAN *et al.* 2003), Infinium® (GUNDERSON 2009), and KASPar™ (SEMAGN *et al.* 2014) assays,

this sequencing approach has become an extremely attractive method for identifying polymorphic markers. To develop a large number of SNPs for genetic mapping, RRLs were constructed and sequenced for PI 163245, PI 224710, and Fla. 8059.

Broadly characterized, gene mapping identifies the relationship between genome sequence and phenotype, allowing for introgression of desired phenotypes via marker assisted selection (MAS) or transgenesis. Genes can be mapped via association mapping or linkage mapping. Association mapping is performed on populations of unrelated individuals, while linkage mapping is based on families or segregating progeny populations usually derived from crosses between inbred lines. Linkage mapping is generally more desirable for mapping genes in crop species since it is possible to design experiments using controlled mapping populations segregating for the trait(s) of interest.

Linkage mapping can be achieved using two approaches. The first linkage mapping studies were performed via marker-based analysis (MBA) (THODAY 1961). In MBA, all individuals in a mapping population are genotyped and phenotyped. This method is particularly useful when multiple traits are being studied, since genotyping and phenotyping all individuals in a population gathers sufficient data for mapping of several traits simultaneously. However, when only a single trait is being studied, trait-based analysis (TBA, a.k.a. selective genotyping) may be more desirable.

While TBA limits the feasible number of traits studied simultaneously, it has several key advantages. Lebowitz *et al.* (1987) discussed three situations where TBA is more useful or practical than traditional MBA. First, when evaluating an $F_2$ or backcross (BC) population for a phenotype in which only part of a population will survive (e.g. disease or abiotic stress), TBA is capable of detecting QTLs by genotyping only a portion

of surviving individuals. Secondly, TBA analysis can be performed on selection lines developed from crosses between inbred parents. Lastly, when costs of genotyping are significantly higher than developing and phenotyping mapping populations, TBA can substantially reduce costs without sacrificing power for QTL detection (LEBOWITZ *et al.* 1987). In fact, the majority of useful data corresponding to QTL detection is obtained from the tails of the phenotypic distribution and it is rarely useful to genotype more than the upper and lower 25% of the population (DARVASI AND SOLLER 1992). Since the objective of this study only concerned a single trait (LB resistance) and genotyping costs were greater than the costs of phenotyping and population development, a TBA approach was selected for mapping LB resistance in PI 163245 (Chapter 4) and PI 224710 (Chapter 5).

## Dissertation Research Objectives

**1.** Perform parent-offspring (P:O) correlation analyses to estimate heritability of LB resistance and calculate response to selection to confirm the utility of LB resistance conferred by *S. pimpinellifolium* accessions PI 163245 (Chapter 2) and PI 224710 (Chapter 3)

**2.** Develop $F_2$ mapping populations, identify a large number of polymorphic SNPs, and perform TBA to delineate genomic regions associated with LB resistance in PI 163245 (Chapter 4) and PI 224710 (Chapter 5)

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# Chapter 2 Genetic characterization and heritability of late blight resistance conferred by the *Solanum pimpinellifolium* accession PI 163245

## Abstract

Late blight (LB), caused by the oomycete, *Phytophthora infestans* (Mont.) de Bary, is one of the most devastating diseases of potato and tomato. The emergence of new aggressive and fungicide resistant *P. infestans* isolates has prioritized identification of new sources of genetic resistance to LB in potato and tomato breeding. A *S. pimpinellifolium* accession, PI 163245, was previously identified as highly resistant to LB in field, greenhouse and detached leaflet screenings. In order to determine the utility of this accession for breeding, the heritability ($h^2$) of LB resistance was estimated in filial progeny derived from hybridizations between PI 163245 and the LB susceptible inbred tomato breeding line Fla.8059. Late blight disease severity (% DS) was measured in $F_2$, $F_3$, and $F_4$ generations in controlled greenhouse screenings. Heritability ($h^2$) was estimated in replicated experiments by $F_2$:$F_3$ and $F_3$:$F_4$ parent-offspring (P:O) correlation analyses, averaging 0.79 and 0.94 respectively. Additionally, the realized heritability ($h^2_R$) measured by response to selection from $F_2$ to $F_4$ generations averaged 0.63. Two methods were utilized to estimate the number of LB resistance genes in PI 163245 and each suggested the involvement of a single resistance locus. The high estimates of $h^2$ and strong response to selection, suggest PI 163245 is a potentially valuable source of LB resistance for tomato. Breeding lines containing LB resistance conferred by PI 163245 are currently being developed in The Pennsylvania State University's tomato breeding program.

## Introduction

Late blight (LB), caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is a highly destructive disease of tomato and potato. The disease notorious for the Irish Potato Famine is capable of destroying susceptible tomato and potato crops within 7-10 days of infection (FOOLAD *et al.* 2008; NOWICKI *et al.* 2012). Late blight is estimated to result in up to 7% yield losses each year in the United States (NOWICKI *et al.* 2012) and is ranked the eighth most important disease of tomato worldwide based on weighted crop average (GAVINO *et al.* 2000). The effects of LB on potato have an even greater impact, resulting in field yield losses of 5% and up to 17% tuber loss in storage (GUENTHNER *et al.* 2001). Economic losses due to LB in potato production alone are estimated at greater than $6 billion annually (HAVERKORT *et al.* 2008; HAVERKORT *et al.* 2009).

Late blight can infect all above ground plant tissue as well as potato tubers. Symptoms of LB consist of dark brown, purple, or black lesions. Severe LB infection eventually results in complete defoliation. *P. infestans* can reproduce sexually and asexually and is a heterothallic organism that requires A1 and A2 mating types for sexual reproduction (JUDELSON 1997). Until the 1980s, only the A1 mating type was found outside of Mexico, preventing sexual reproduction and restricting its evolutionary potential (FRY AND GOODWIN 1997). However, immigration of the A2 mating type to Europe and the United States in the 1980s and early 1990s has increased the potential for generation of new and potentially more aggressive clonal lineages (HOHL AND ISELIN 1984; DEAHL *et al.* 1991). Fortunately, in the United States only two sexual populations of *P. infestans* have been detected (GAVINO *et al.* 2000; DANIES *et al.* 2014). However,

the frequency of sexual *P. infestans* populations has increased considerably in Europe (YUEN AND ANDERSSON 2013).

Several factors contribute to the destructiveness of LB. *P. infestans* has the ability to rapidly destroy susceptible potato and tomato crops within several days of colonization. Additionally, initial symptoms of LB are difficult to identify and detection is often too late for effective treatment with fungicides. Late blight spreads extremely rapidly, with each lesion producing as many as 300,000 sporangia per day. Finally, the short asexual life cycle of *P. infestans* can occur in fewer than five days (FRY AND GOODWIN 1997). The *P. infestans* genome structure also contributes to the effectiveness of the pathogen, facilitating rapid breakdown of host resistance. While important housekeeping genes are contained in conserved blocks, the majority of apoplastic and cytoplasmic effector genes occupy repetitive genomic regions with relatively low gene densities, resulting in frequent non-allelic recombination and tandem gene duplication events (HAAS *et al.* 2009). The combination of these factors make LB extremely difficult to effectively manage.

Prior to the development of more aggressive clonal lineages and fungicide resistant populations, LB was managed primarily through good cultural practices and frequent fungicide applications (FOOLAD *et al.* 2008). Cultural practices which reduce the incidence of LB, include crop rotation and fallow of potato fields, destruction of inoculum sources such as potato cull piles, and early detection, elimination, and treatment of infected plants (FOOLAD *et al.* 2008). However, these management strategies are becoming less effective due to the development of new *P. infestans* clonal lineages and fungicide resistant populations. Especially concerning is the occurrence *P. infestans*

populations containing resistance to phenylamides, one of the most effective classes of systemic fungicides (GISI AND COHEN 1996; GOODWIN *et al.* 1996). Currently, a combination of protectant and translaminar fungicides are commonly used in managing LB infection. However, the environmental and economic costs associated with frequent fungicide applications emphasize the importance of cultivar development with durable genetic LB resistance.

Identification of LB resistance has only recently become a priority in tomato breeding, and consequently relatively few resistance genes are available in commercial cultivars. The first LB resistance gene reported in tomato was the dominant gene *Ph-1*, identified in *S. pimpinellifolium* accessions West Virginia 19 and 731 (BONDE AND MURPHY 1952; GALLEGLY AND MARVEL 1955). *Ph-1* was mapped to the long arm of chromosome 7 (PEIRCE 1971). However, *Ph-1* only confers resistance against *P. infestans* race T-0 and is ineffective against the predominant *P. infestans* race, T-1 (PEIRCE 1971). Consequently, *Ph-1* has limited viability in tomato breeding (FOOLAD *et al.* 2008). The *Ph-2* resistance gene was identified in the *S. pimpinellifolium* accession West Virginia 700 (GALLEGLY AND MARVEL 1955). *Ph-2* was mapped to a distal 8.4 cM region on the long arm of chromosome 10 (MOREAU *et al.* 1998). Conferring partial resistance, *Ph-2* is generally associated with slowed rates of disease progression (MOREAU *et al.* 1998; FOOLAD *et al.* 2008). Unfortunately, tomato cultivars containing only *Ph-2* are not sufficiently resistant when exposed to aggressive *P. infestans* clonal lineages without pyramiding additional LB resistance genes (GOODWIN *et al.* 1995; BLACK *et al.* 1996; FOOLAD *et al.* 2008). However, a combination of *Ph-2* and *Ph-3* LB resistance genes provides high levels of resistance against many *P. infestans* clonal lineages, and tomato

cultivars containing both resistance genes are commercially available (GARDNER AND PANTHEE 2010b; GARDNER AND PANTHEE 2010a; PANTHEE AND GARDNER 2010). *Ph-3* is the strongest single source of LB resistance in tomato and was identified in the *S. pimpinellifolium* accession L3708 and was mapped to the long arm of chromosome 9 (CHUNWONGSE *et al.* 2002). *Ph-3* is the only tomato LB resistance gene fine mapped and cloned, and encodes a CC-NBS-LRR class protein (ZHANG *et al.* 2013; ZHANG *et al.* 2014).

Additional LB resistant *S. pimpinellifolium* accessions have been reported, but genetic resistance obtained from these sources has not been released in any commercial cultivars. The source of *Ph-3*, L3708 contains a second genomic region, *qPh2.1*, associated with LB resistance which was mapped to chromosome 2 (CHEN *et al.* 2014). Late blight resistance was also reported in the *S. pimpinellifolium* accession, L3707 and is likely controlled by two loci (IRZHANSKY AND COHEN 2006). A particularly promising source of resistance, *S. pimpinellifolium* accession PI 270443, displays high levels of LB resistance statistically similar to *Ph-2* + *Ph-3* combined. PI 270443 was further characterized and two genomic loci associated with LB resistance were mapped to chromosomes 1 and 10. Breeding efforts to implement these resistance genes in tomato breeding lines are ongoing with good success (MERK *et al.* 2012; MERK AND FOOLAD 2012).

In addition to *S. pimpinellifolium*, LB resistance genes were identified in wild tomato species *S. habrochaites* and *S. pennellii* (BROUWER *et al.* 2004; BROUWER AND ST. CLAIR 2004; SMART *et al.* 2007; ABREU *et al.* 2008; LI *et al.* 2011). *S. habrochaites* accession LA2099 confers race non-specific LB resistance and QTLs were mapped on all

12 tomato chromosomes (BROUWER *et al.* 2004). The strongest of these QTLs were fine mapped to chromosomes 4 (*lb4*), 5 (*lb5b*), and 11 (*lb11b*) (BROUWER AND ST. CLAIR 2004). However, implementation of these genes using traditional breeding methods including marker assisted selection (MAS) were unsuccessful due to linkage drag, resulting in undesirable horticultural characteristics including poor plant and canopy shape, low yield, small fruit, and late maturity (BROUWER AND ST. CLAIR 2004). Late blight resistance was also identified and mapped in the *S. habrochaites* accession LA1777, with five QTLs explaining most of the resistance (LI *et al.* 2011). Four of the QTLs co-localized with those previously mapped in LA2099 on chromosomes 4, 7, 8, and 12, although a potentially novel QTL on chromosome 4 (*Rlbq*4b) was identified (LI *et al.* 2011). Additionally, LB resistance was reported in the *S. habrochaites* accession BGH6902 (ABREU *et al.* 2008). However, Abreu *et al.* estimated the involvement of as many as 28 genes, limiting its potential usefulness in breeding. The *S. pennellii* accession LA716 reportedly contains a QTL on chromosome 6 associated with LB resistance, though this QTL is tightly linked with the self-pruning (*Sp*) locus and the authors suggest indeterminate growth habit could be responsible for the observed LB resistance, since individuals may have been outgrowing the rate of LB infection (SMART *et al.* 2007).

Previously, 67 *S. pimpinellifolium* accessions were evaluated for LB resistance in field, greenhouse, and detached leaflet analysis (DLA) and twelve accessions displaying resistance similar to *Ph-2* + *Ph-3* combined were identified (FOOLAD *et al.* 2014b) (FOOLAD *et al.* 2014a). Among these accessions, PI 163245 was highly resistant to LB in all experiments and was resistant to several *P. infestans* isolates from clonal lineages US-13, US-14, and US-23 (FOOLAD *et al.* 2014b). Molecular markers associated with *Ph-2*

and *Ph-3* suggested neither of these genes were responsible for the observed resistance, although genetic mapping is required to confirm the novelty of LB resistance in PI 163245.

Determining the heritability ($h^2$) of a trait is highly useful, as it enables prediction of response to selection and can help estimate the efficiency of potential mapping studies. Heritability provides a measure of the proportion of total phenotypic variance attributable solely to genetic variance. In theory, heritability is population specific, but heritabilities are often similar between populations or even species (VISSCHER *et al.* 2008). Heritability may be measured in the broad-sense (additive + dominance variance) or in the narrow-sense (additive variance). Generally, an estimate of heritability in the narrow-sense is more useful, since dominance effects have less value in plant breeding. It is also possible to determine the realized heritability ($h^2_R$), by calculating the ratio of cumulative selection response ($R$) to cumulative selection differential ($S$).

Few studies have examined the $h^2$ of LB resistance in tomato. Previously, Abreu *et al*. (2008) evaluated heritability in the *S. habrochaites* accession BGH6902 using generation means analysis, and determined that $h^2$ was extremely low (0.09). In contrast, Merk and Foolad (2012) utilized parent-offspring (P:O) correlation analysis, which estimated the $h^2$ of LB resistance conferred by the *S. pimpinellifolium* accession PI 270443 was 0.86, indicating the trait was highly heritable. When $h^2$ estimates are low, selections for the desired trait are likely to yield limited success. Consequently, estimating $h^2$ can help determine whether breeding efforts are warranted.

In order to estimate $h^2$ of LB resistance conferred by PI 163245, a P:O correlation analysis approach was selected. In crop species, P:O correlation analysis usually provides

a reliable estimate of response to selection (FALCONER AND MACKAY 1996). However, if dominance genetic variance is large, estimates of $h^2$ can be overestimated in early generations. To account for this possibility, $h^2$ was estimated in multiple P:O generations for this study. These estimates of heritability were further validated by calculating $h^2_R$. The purpose of this study was to determine the utility of PI 163245 for breeding LB resistance in tomato. Consequently, $h^2$ was estimated using $F_2$:$F_3$ and $F_3$:$F_4$ P:O correlation analyses and compared to the $F_4$ $h^2_R$ based on selections in $F_2$ and $F_3$ generations. Additionally, two methods were employed to estimate the number of LB resistance genes.

## Materials and Methods

### Plant Material

The LB susceptible tomato breeding line Fla.8059 (pistillate parent) was hybridized with the LB resistant *S. pimpinellifolium* accession PI 163245 (staminate parent). Fla.8059 produces large, ultrafirm fruit with high lycopene and good flavor (SCOTT *et al.* 2008). However, Fla.8059 is highly susceptible to LB. Original seed from Fla.8059 was provided by J.W. Scott, University of Florida, Gulf Coast Research Education Center, Wimauma, FL, USA. The LB resistant accession, PI 163245, is an inbred line and displayed strong LB resistance in field, greenhouse, and detached leaflet experiments (FOOLAD *et al.* 2014a; FOOLAD *et al.* 2014b). Although highly resistant to LB, PI 163245 has undesirable horticultural characteristics including large and indeterminate plant size, somewhat exerted stigmata, and small yellow fruit. PI 163245 seed was provided by the USDA Plant Genetic Resources Unit (PGRU), Geneva, NY, USA. Hybrid $F_1$ progeny were self-pollinated for $F_2$ seed. A large $F_2$ (n = 560) population

was grown and evaluated for LB resistance and highly resistant (n = 63) and susceptible (n = 36) individuals were retained, grown to maturity, and self-pollinated. $F_3$ (progeny of $F_2$) and $F_4$ (progeny of $F_3$) families were developed and evaluated for disease resistance to estimate heritability and calculate response to selection (described below). In all experiments, parental, $F_1$ and control lines were included. Control lines consisted of NC 84173 (LB susceptible), New Yorker (*Ph-1*), NC 63EB (*Ph-2*), NC 870 (*Ph-3*), and NC 03220 (*Ph-2* + *Ph-3*). Seed for all control lines was provided by R.G. Gardner, North Carolina State University, Mills River, NC, USA.

**Inoculum preparation**

The highly aggressive and widely prevalent *P. infestans* clonal lineage US-23, race T-1, mating type A1, was used for all experiments. An isolate from US-23 was selected due to its widespread occurrence throughout the Northeastern United States and its highly aggressive rates of infection in both tomato and potato (GUGINO AND FOOLAD 2013; FOOLAD *et al.* 2014b; GUGINO *et al.* 2014). The isolate used for this study, RS2009T1, was originally collected in Rock Springs, PA in 2009 from a commercial tomato field. RS2009T1 was cultured in sterile 100 × 15 mm petri dishes on susceptible tomato leaflets placed abaxial (lower) side up. A thin layer of water agar (1.7%) lined the petri dish lid in order to maintain high humidity. The LB infected tomato leaflets were incubated on a 12 hour photoperiod for 7-11 days at 14-16 ºC and 100% humidity. Inoculum was prepared by placing infected leaflets in 4 ºC distilled water and incubating for 1 hour at 4 ºC to facilitate zoospore release. The suspension was briefly vortexed to dislodge sporangia and filtered through cheesecloth to remove leaf debris. The inoculum was adjusted to 10,000 sporangia/mL using a haemocytometer and light microscope.

**$F_2$ disease evaluation**

A large $F_2$ population (n = 560), parental lines (PI 163245 and Fla.8059), $F_1$ progeny, and resistant and susceptible control genotypes were grown in 72-cell flats in an isolated and environmentally controlled greenhouse. Parental, $F_1$, and control lines consisted of four replications of six individuals and were positioned on opposite ends and sides of the greenhouse compartment. When the plants were approximately six weeks old, high pressure foggers were initiated to increase the relative humidity to 95-100% and the temperature was reduced to 16-18 ºC. Clear plastic was draped around each bench to prevent direct accumulation of water on the plants. Six hours later, high pressure foggers were temporarily halted and the plants were lightly misted with water. After 30 minutes, *P. infestans* inoculum was sprayed uniformly over all plants at a concentration of 10,000 sporangia/mL and a volume of approximately 1 L per 1,000 plants. The same concentration and volume of inoculum was applied a second time 30 minutes later and the high pressure foggers were reinitiated for the remainder of the experiment. Blackout curtains were used for the first 24 hours to reduce ambient light and suppress hypersensitive and salicylic acid defense responses (GRIEBEL AND ZEIER 2008; RODEN AND INGLE 2009).

Seven days following infection, the plants were evaluated for disease severity (% DS) on a scale of 0-100%. Each $F_2$ individual was assigned a % DS score, where 0% indicated no affected tissue, and 100% indicated no remaining healthy tissue or complete defoliation. The parental, $F_1$ progeny, and control lines were assigned a mean % DS by visually estimating the average % DS for each replicate. From the 560 $F_2$ plants, 63 of the

most resistant (% DS <20) and 36 of the most susceptible (% DS >85) were retained, grown to maturity, and self-pollinated for production of $F_3$ progeny families.

**$F_3$ disease evaluations**

The $F_3$ progeny families were evaluated in four separate experiments (I, II, III, IV). Many $F_3$ families were included in all four experiments, however due to insufficient seed some families were included in fewer. From the 99 distinct $F_3$ progeny families, 31 were included in all experiments, 26 were evaluated in three experiments, 18 were screened in two experiments, and 24 families only produced sufficient seed for inclusion in one experiment. Screenings were conducted similarly as the $F_2$ experiment, previously described. Due to insufficient space in a single greenhouse section, two adjacent and similar compartments were used for experiments I, II, and III, each compartment containing one of two replicates and totaling 10-12 plants combined. In Experiment IV, both replicates were contained in the same greenhouse compartment and separated on opposite benches. Experiment I consisted of 45 resistant and 19 susceptible families, experiment II contained 39 resistant and 20 susceptible families, experiment III had 52 resistant and 24 susceptible families, and experiment IV was composed of 49 resistant and 14 susceptible families. Experiments I and II were evaluated six and seven days following inoculation respectively. Experiments III and IV were each evaluated five days after inoculation. Seedlings were approximately six weeks old when inoculation was performed for each disease evaluation. Parental and control genotypes excluding NC 84173 were included in all experiments. NC 84173 and $F_1$ progeny were only included in experiments I and IV. Each $F_3$ individual was evaluated and assigned a single % DS and the scores were averaged for each family. Parental, $F_1$, and control genotypes were

assigned a mean % DS for each replicate. In experiment IV, the most resistant individual from each replicate of the resistant class $F_3$ progeny families and the most susceptible, surviving individual from each of the susceptible class $F_3$ progeny family replicates were selected. These plants were grown to maturity and self-pollinated to produce $F_4$ progeny families to perform $F_3$:$F_4$ P:O correlation analysis and measure response to selection.

**$F_4$ disease evaluations**

$F_4$ progeny families were screened at two separate times and are considered two experiments (I and II). Due to poor germination and some damage caused by thrips infection, some families were excluded from experiment II. Consequently, experiment I consisted of 47 resistant class and 24 susceptible class $F_4$ progeny families, while experiment II consisted of 30 resistant and 22 susceptible $F_4$ progeny families. For each $F_4$ family, two replicates totaling 10-12 individuals were grown on opposite benches in the same greenhouse section. Inoculations and disease evaluations were performed as previously described when the plants were approximately six weeks old. Experiment I was evaluated seven days following inoculation and experiment II was evaluated after eight days. Unlike the $F_2$ and $F_3$ experiments, the parental, $F_1$, and control lines were evaluated similarly to the $F_4$ progeny families and each individual plant was assigned a % DS before the average was calculated.

**Data analysis**

Estimates of heritability and number of resistance loci were obtained from experiments conducted in the $F_2$, $F_3$, and $F_4$ generations. The heritability ($h^2$) of resistance was estimated by P:O correlation analysis for $F_2$:$F_3$ and $F_3$:$F_4$ using the following equations:

$$h^2(\mathrm{F}_{2:3}) = r_{\mathrm{F2:F3}} = \frac{Cov_{F3,F2}}{(V_{\overline{F3}}V_{F2})^{1/2}} = \frac{V_A + 1/2\,V_D}{[(V_A + 1/4\,V_D + 1/n\,V_E)(V_A + V_D + V_E)]^{1/2}}$$

and

$$h^2(\mathrm{F}_{3:4}) = r_{\mathrm{F3:F4}} = \frac{Cov_{F4,F3}}{(V_{\overline{F4}}V_{F3})^{1/2}} = \frac{3/2\,V_A + 3/8\,V_D}{[(3/2\,V_A + 3/16\,V_D + 1/n\,V_E)(3/2\,V_A + 3/4\,V_D + V_E)]^{1/2}},$$

where *r* is the correlation coefficient, *Cov* is the covariance between parent and progeny generations, $V_{F2}$ is the variance of the $F_2$ parental generation, $V_{\overline{F3}}$ and $V_{\overline{F4}}$ are the variances among $F_3$ and $F_4$ progeny families respectively, $V_A$, $V_D$, and $V_E$ are the additive, dominance, and environmental variances, and *n* is the number of individuals in each of the $F_3$ or $F_4$ progeny families (FALCONER AND MACKAY 1996). The standard errors (*SE*) of the $h^2$ estimates were obtained by the formulae:

$h^2(\mathrm{F}_{2:3}) = [(1\text{-}r^2_{\mathrm{F2:F3}})/(n\text{-}2)]^{1/2}$ and $h^2(\mathrm{F}_{3:4}) = [(1\text{-}r^2_{\mathrm{F3:F4}})/(n\text{-}2)]^{1/2}$,

where *n* is equal to the number of families used to estimate $h^2$. The realized heritability ($h^2_R$) for LB resistance based on two rounds of selection was measured by response to selection from $F_2$ to $F_4$ generations by the equation:

$$h^2_R = \frac{R_{F2:F3} + R_{F3:F4}}{S_{F2:F3} + S_{F3:F4}},$$

where *R* is the response to selection [change in mean % DS between parent (e.g. $F_2$ or $F_3$) and progeny generations (e.g. $F_3$ or $F_4$)], and *S* is the selection differential (difference between the mean of selected resistant individuals and overall mean of the parental population prior to selection) (FALCONER AND MACKAY 1996). When calculating $h^2_R$, only resistant class progeny families were included in analysis for $F_3$ and $F_4$ generations.

For calculating the number of loci involved in conferring LB resistance, two methods were employed. The first method compared the proportion of $F_2$ individuals

exhibiting % DS similar to the homozygous susceptible parent to the expected Mendelian ratio of $(¼)^n$, where n is equal to the minimum number of resistance loci in the $F_2$ population. The second method utilized the equation:

$$n = \frac{(m_1 - m_2)^2}{8(V_{F2} - V_{F1})},$$

where $m_1$ and $m_2$ are the average % DS of the two parental lines (Fla. 8059 and PI 163245) and $V_{F2}$ and $V_{F1}$ are the variances in disease response in the $F_2$ and $F_1$ generations, respectively (WRIGHT 1952; FALCONER AND MACKAY 1996).

Mean comparisons of the parental, $F_1$, and resistant and susceptible control lines were calculated using Tukey's HSD test.

## Results

### Disease response of parental, $F_1$, and control lines

The % DS for all parental, $F_1$, and control genotypes are presented in Table 2-1. The % DS of the *S. pimpinellifolium* accession, PI 163245, was low across all experiments, averaging 16.5% DS, and no replicate averaged >40% DS. The % DS was not statistically different from NC 63EB (19.6% DS) or NC 870 (10.3% DS) containing *Ph-2* and *Ph-3* respectively. Although PI 163245 slightly outperformed NC 63EB, it did not consistently provide as high levels of resistance as NC 870. NC 03220 (*Ph-2* + *Ph-3*) had the lowest % DS, averaging 4.6% DS. In all experiments, Fla. 8059 was highly susceptible to LB, averaging 92.4% DS. No Fla.8059 replicate in any experiment averaged <50% DS. Fla.8059 was statistically similar to NC 81473 (94.5% DS) and to New Yorker (93.4% DS). The $F_1$ % DS was 50.4% and ranged from 25-80% DS, potentially indicating that LB resistance conferred by PI 163245 is codominant.

**Table 2-1** Late blight disease severity (% defoliation/foliar disease symptoms ± SD) for susceptible (Fla. 8059) and resistant (PI 163245) parents, $F_1$ progeny, control lines, and $F_2$, $F_3$, and $F_4$ experimental populations.

| | Genotype | Number of Plants or Families | % DS[1] | Range (%DS) |
|---|---|---|---|---|
| | $P_1$ (Fla. 8059) | 240 | 92.4 ± 8.6[a] | 50-100 |
| | $P_2$ (PI 163245) | 221 | 16.5 ± 9.4[cd] | 5-40 |
| | $F_1$ | 100 | 50.4 ± 12.7[b] | 25-80 |
| | NC 84173 | 120 | 94.5 ± 4.5[a] | 85-100 |
| | New Yorker (*Ph-1*) | 240 | 93.4 ± 5.9[a] | 70-100 |
| | NC 63EB (*Ph-2*) | 236 | 19.6 ± 11.2[c] | 5-60 |
| | NC 870 (*Ph-3*) | 207 | 10.3 ± 4.5[de] | 5-20 |
| | NC 03220 (*Ph-2* + *Ph-3*) | 211 | 4.6 ± 2.6[e] | 0-10 |
| | $F_2$ population | 560 | 51.9 ± 30.2 | 0-100 |
| **$F_2$:$F_3$ Experiment I** | $F_2$ selected individuals (resistant class) | 45 | 7.9 ± 4.8 | 0-20 |
| | $F_2$ selected individuals (susceptible class) | 19 | 91.2 ± 2.0 | 90-95 |
| | $F_3$ progeny families (resistant class) | 45 | 30.0 ± 24.9 | 2-100 |
| | $F_3$ progeny families (susceptible class) | 19 | 65.8 ± 29.6 | 5-100 |
| **$F_2$:$F_3$ Experiment II** | $F_2$ selected individuals (resistant class) | 39 | 7.5 ± 4.5 | 0-20 |
| | $F_2$ selected individuals (susceptible class) | 20 | 90.4 ± 2.5 | 85-95 |
| | $F_3$ progeny families (resistant class) | 39 | 30.5 ± 28.5 | 2-100 |
| | $F_3$ progeny families (susceptible class) | 20 | 80.9 ± 25.6 | 5-100 |
| **$F_2$:$F_3$ Experiment III** | $F_2$ selected individuals (resistant class) | 52 | 7.7 ± 4.7 | 0-20 |
| | $F_2$ selected individuals (susceptible class) | 24 | 90.0 ± 2.9 | 85-97 |
| | $F_3$ progeny families (resistant class) | 52 | 28.9 ± 28.8 | 1-100 |
| | $F_3$ progeny families (susceptible class) | 24 | 85.67 ± 23.6 | 3-100 |
| **$F_2$:$F_3$ Experiment IV** | $F_2$ selected individuals (resistant class) | 49 | 8.0 ± 4.9 | 0-20 |
| | $F_2$ selected individuals (susceptible class) | 14 | 90.0 ± 2.3 | 85-97 |
| | $F_3$ progeny families (resistant class) | 49 | 28.9 ± 23.9 | 0-99 |
| | $F_3$ progeny families (susceptible class) | 14 | 85.7 ± 26.6 | 5-100 |
| **$F_3$:$F_4$ Experiment I** | $F_3$ selected individuals (resistant class) | 47 | 4.4 ± 2.6 | 2-15 |
| | $F_3$ selected individuals (susceptible class) | 24 | 92.5 ± 4.6 | 85-100 |
| | $F_4$ progeny families (resistant class) | 47 | 13.2 ± 19.6 | 0-100 |
| | $F_4$ progeny families (susceptible class) | 24 | 74.7 ± 28.9 | 5-100 |
| **$F_3$:$F_4$ Experiment II** | $F_3$ selected individuals (resistant class) | 30 | 4.9 ± 3.4 | 2-15 |
| | $F_3$ selected individuals (susceptible class) | 22 | 92.4 ± 4.6 | 85-100 |
| | $F_4$ progeny families (resistant class) | 30 | 16.3 ± 15.8 | 1-100 |
| | $F_4$ progeny families (susceptible class) | 22 | 83.1 ± 21.4 | 5-100 |

[1]Mean comparisons of parental, $F_1$, and control lines were determined using Tukey's HSD test ($P \leq 0.05$) and are denoted by superscripts (a-e).

## $F_2$ disease response

The $F_2$ population (n = 560) averaged 51.9% DS and ranged from 0-100% DS. The Shapiro-Wilk test for normality indicated non-normal distribution ($P < 0.001$) and the population was skewed slightly towards susceptibility (skewness = -0.19), moderately

resembling a bimodal distribution. In the $F_2$ population, 319 individuals displayed disease response ≥50% DS while 241 individuals exhibited <50% DS (Fig. 2-1). Selected resistant class $F_2$ individuals averaged 7.9 ± 4.8% DS and ranged from 0-20% DS. The susceptible class $F_2$ individuals averaged 91.2% DS, ranging from 90-95% DS (Table 2-1, Figs. 2-2, 2-3, 2-4, 2-5).

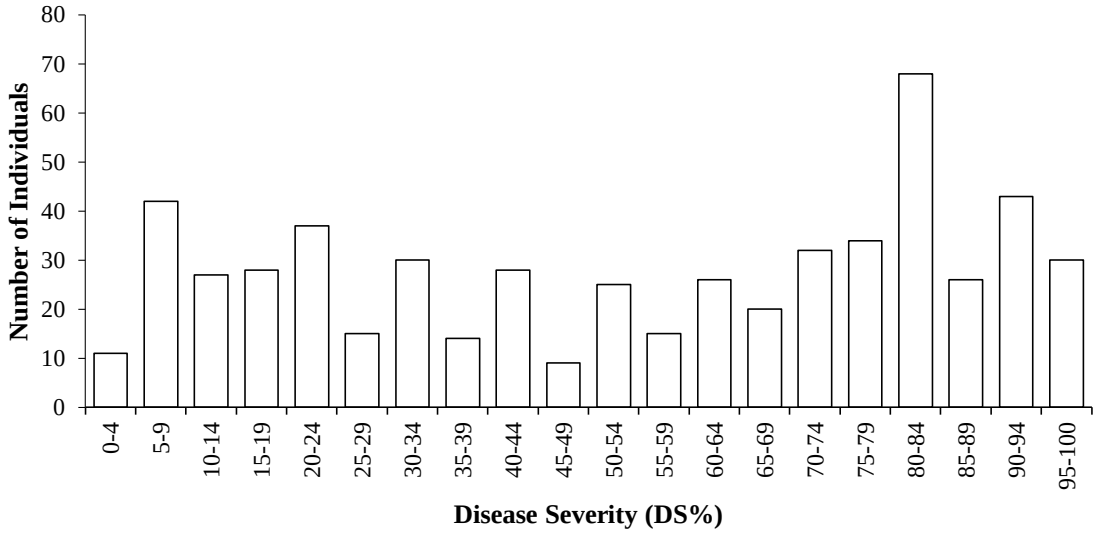


**Figure 2-1** Frequency distribution of disease severity (% DS) of the $F_2$ population (n = 560). Foliar % DS was measured as a percentage of foliar disease symptoms/defoliation.

### $F_2$:$F_3$ parent-offspring correlation analyses

$F_3$ progeny families were screened four separate times, and $h^2$ was calculated individually for each experiment. The results were generally consistent across all four experiments. The mean resistant class $F_3$ progeny families ranged from 19.7-30.5% DS and the susceptible class $F_3$ progeny families averaged from 65.8-85.7% DS (Table 2-1). Each experiment consisted of two replicates for each progeny family and since correlations were high ($r > 0.67$, $P < 0.001$), replicates were pooled within each experiment to estimate $h^2$. The $F_3$ progeny populations were skewed towards resistance

and susceptibility. Generally, resistant class progeny families resembled their resistant $F_2$ parents and susceptible progeny resembled their LB susceptible $F_2$ parents (Figs. 2-2, 2-3, 2-4, 2-5). However, the mean % DS of eight resistant class families was >60%, while seven of the susceptible class families averaged <60% DS in at least one experiment. Estimates of $h^2$ based on P:O correlation analysis ranged from 0.76-0.81 suggesting heritability of PI 163245 LB resistance is moderately high (Table 2-2). The selected resistant and susceptible $F_3$ individuals (parents of $F_4$ progeny families) averaged 4.4-4.9% DS and 92.4-92.5% DS respectively for each experiment.

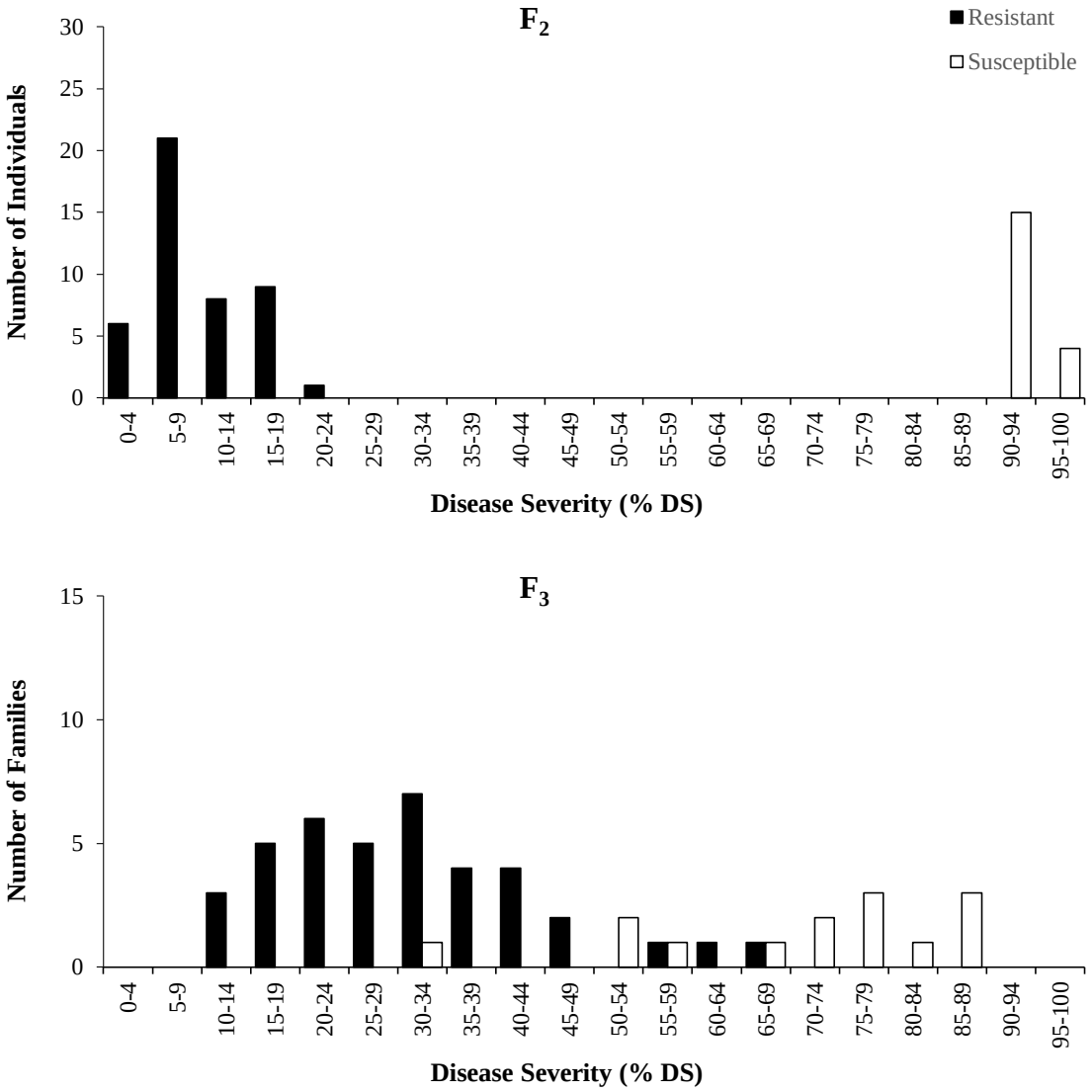


**Figure 2-2** Experiment I frequency distributions of disease severity (% DS) for selected resistant and susceptible $F_2$ individuals (top) and mean % DS of $F_3$ progeny families (bottom). Foliar % DS was measured as a percentage of whole plant foliar disease symptoms/defoliation.

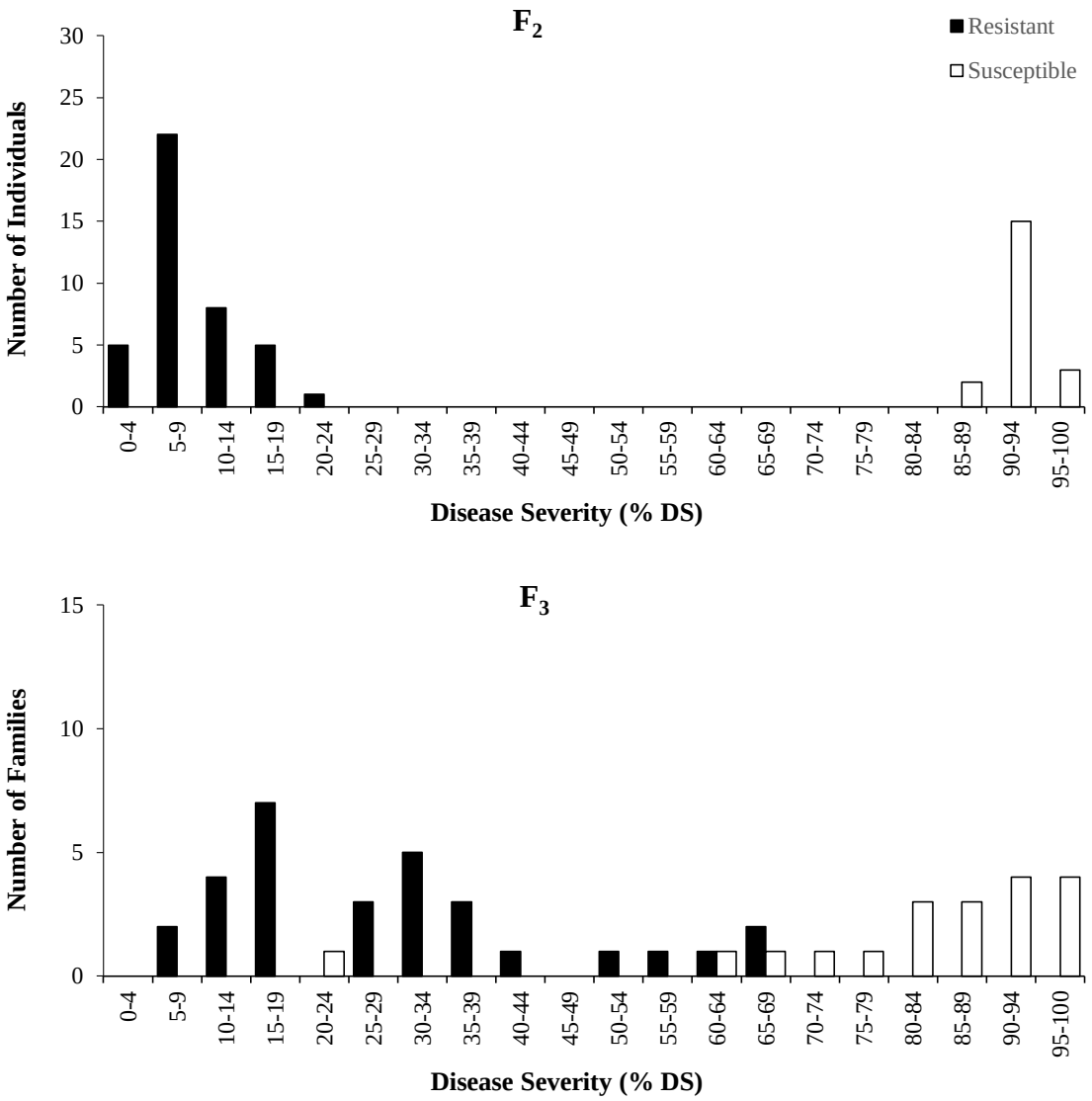


**Figure 2-3** Experiment II frequency distributions of disease severity (% DS) for selected resistant and susceptible $F_2$ individuals (top) and mean % DS of $F_3$ progeny families (bottom). Foliar % DS was measured as a percentage of whole plant foliar disease symptoms/defoliation.

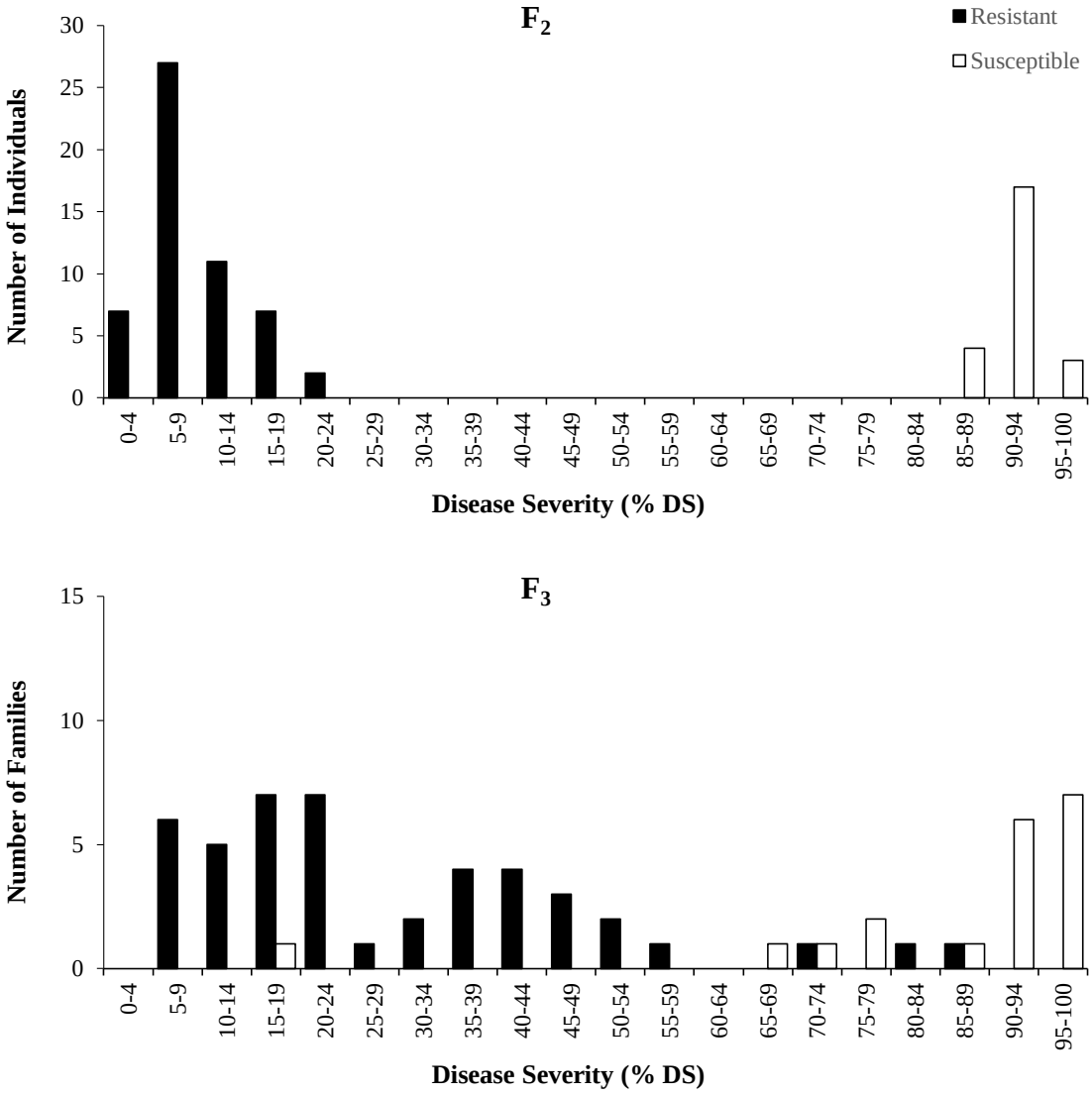


**Figure 2-4** Experiment III frequency distributions of disease severity (% DS) for selected resistant and susceptible $F_2$ individuals (top) and mean % DS of $F_3$ progeny families (bottom). Foliar % DS was measured as a percentage of whole plant foliar disease symptoms/defoliation.

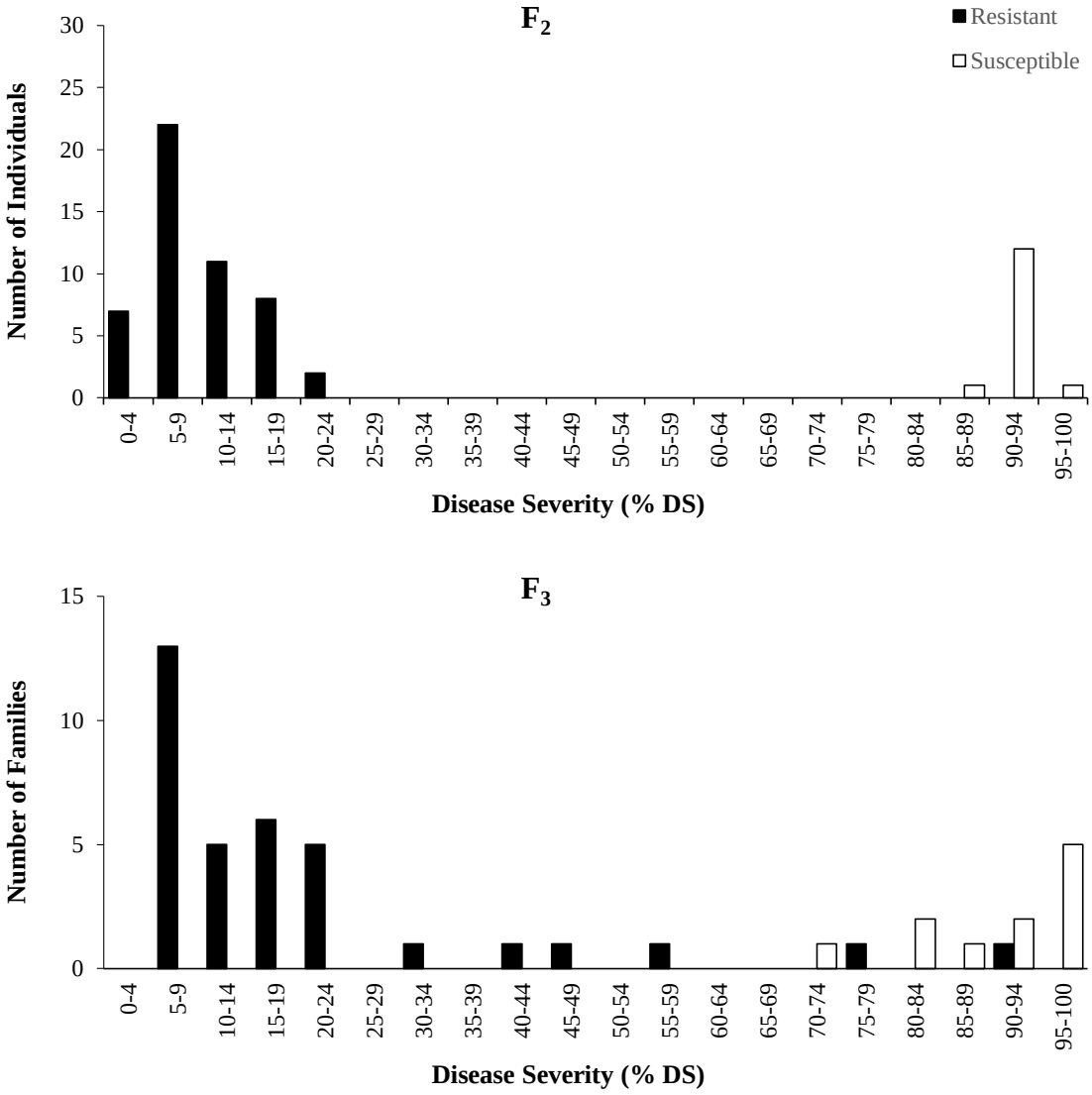


**Figure 2-5** Experiment IV frequency distributions of disease severity (% DS) for selected resistant and susceptible $F_2$ individuals (top) and mean % DS of $F_3$ progeny families (bottom). Foliar % DS was measured as a percentage of whole plant foliar disease symptoms/defoliation.

**$F_3$:$F_4$ parent-offspring correlation analyses**

The $F_4$ progeny families were screened in two separate experiments, and in each instance results were similar. For all $F_4$ experiments, correlation between the two replicates was high ($r > 0.91$, $P < 0.001$) and replicates were pooled within each experiment to estimate $h^2$. In both experiments, the $F_4$ screening populations were skewed towards susceptibility and resistance (Figs. 2-6, 2-7). The resistant class $F_4$ progeny families averaged 13.2% DS and 16.3% DS in experiments I and II respectively. The disease pressure appeared slightly lower in experiment I as susceptible class $F_4$ progeny families averaged 74.7% DS while in experiment II susceptible class $F_4$ progeny families averaged 83.1% DS (Table 2-1). The average % DS for nearly all $F_4$ progeny families resembled their respective $F_3$ parents in both experiments. Only the average % DS for one resistant class $F_4$ progeny family was considerably more susceptible than its $F_3$ parent, while five susceptible class families were markedly more resistant than their $F_3$ parents in one or more experiments. Estimates of $h^2$ using P:O correlation analyses indicated the LB resistance was highly heritable and estimates of 0.91 and 0.97 were obtained for experiments I and II respectively (Table 2-2).

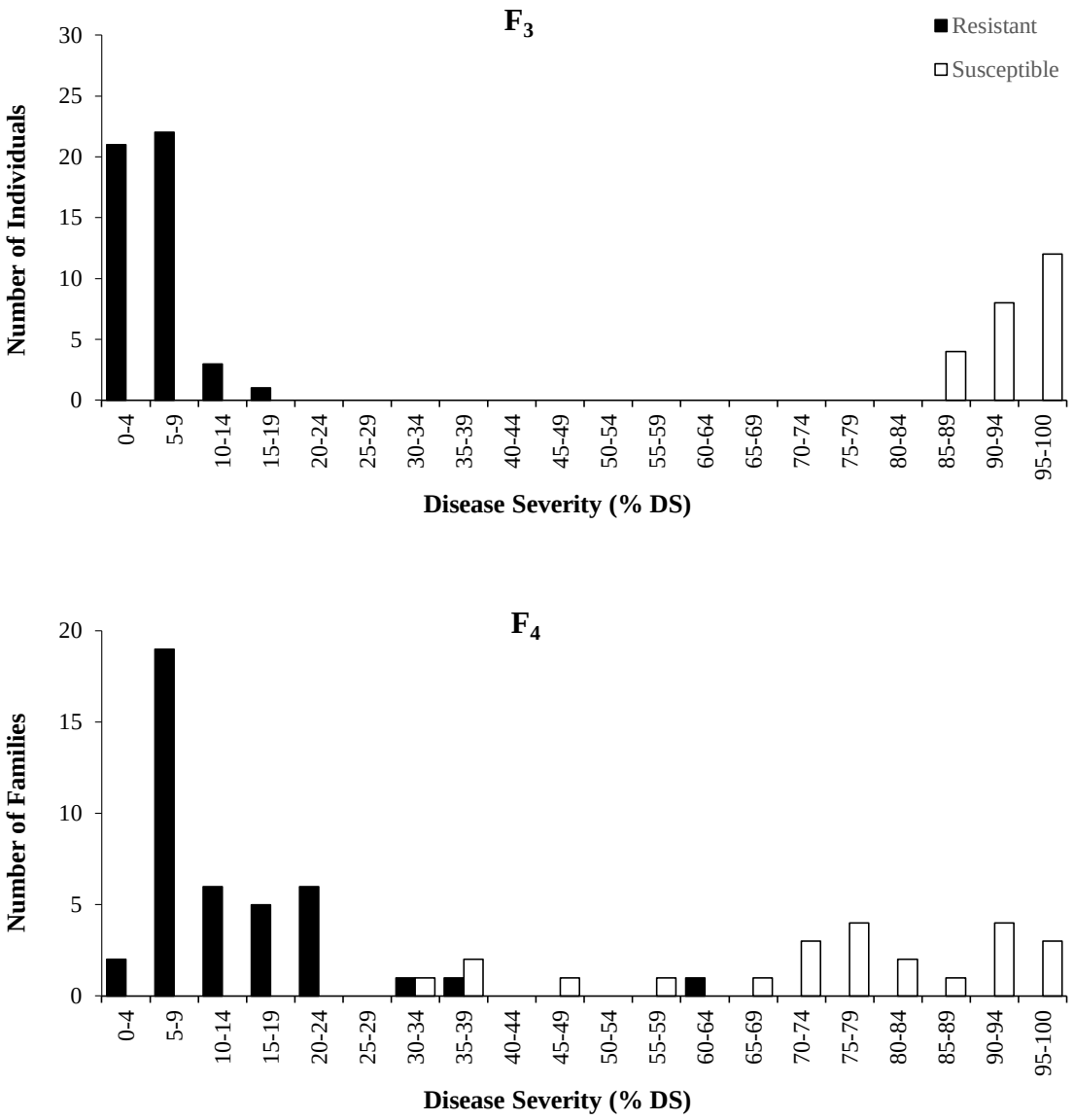


**Figure 2-6** Experiment I frequency distributions of disease severity (% DS) for selected resistant and susceptible $F_3$ individuals (top) and mean % DS of $F_4$ progeny families (bottom). Foliar % DS was measured as a percentage of whole plant foliar disease symptoms/defoliation.

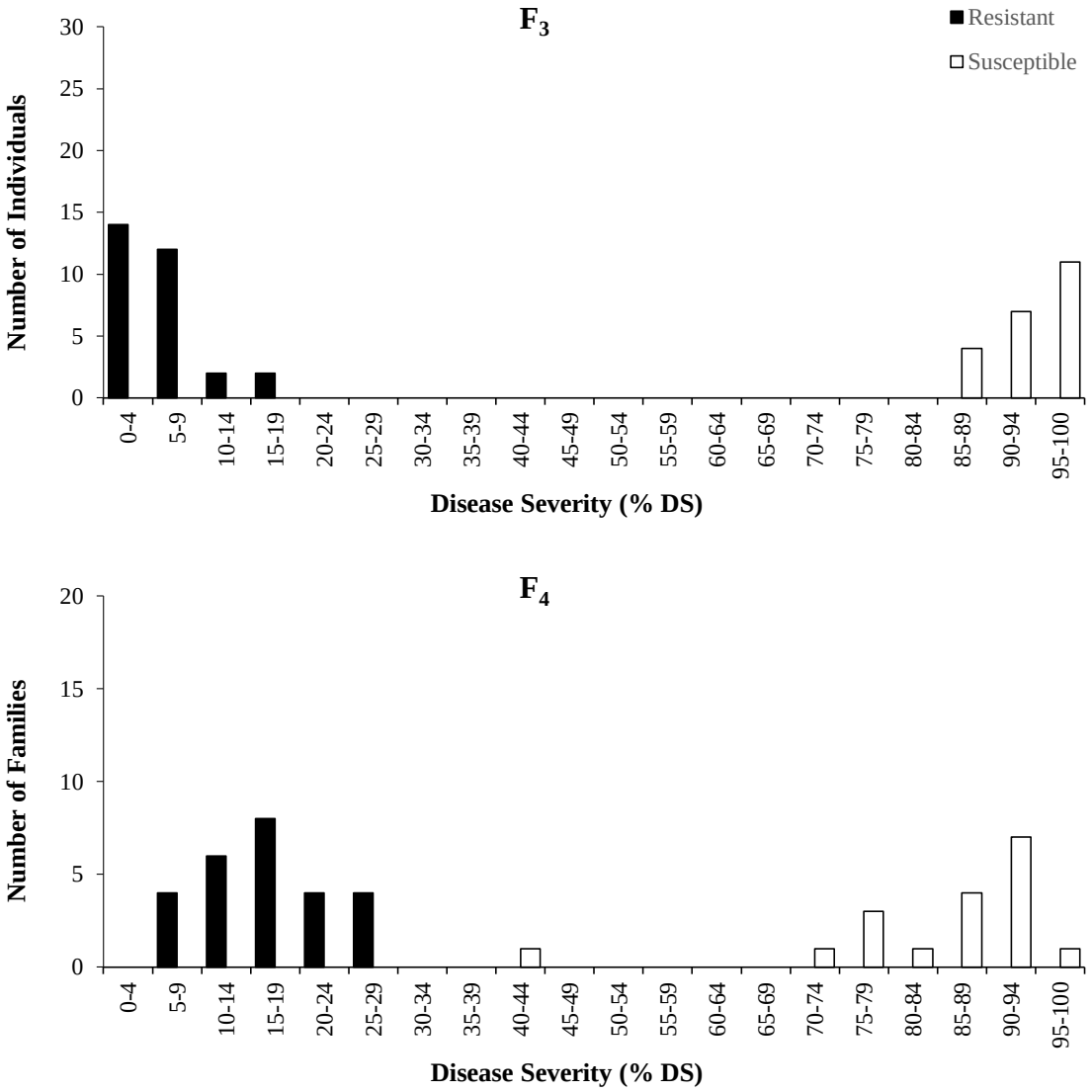


**Figure 2-7** Experiment II frequency distributions of disease severity (% DS) for selected resistant and susceptible $F_3$ individuals (top) and mean % DS of $F_4$ progeny families (bottom). Foliar % DS was measured as a percentage of whole plant foliar disease symptoms/defoliation.

## Realized heritability

In addition to estimates of $h^2$ obtained by P:O correlation analyses, the realized heritability ($h^2_R$) was determined in the $F_4$ generation based on selections in the $F_2$ and $F_3$ generations. Since $F_4$ progeny were evaluated twice, two measures of $h^2_R$ were obtained. The results were similar in both experiments, though slightly higher in experiment I ($h^2_R$ = 0.66) than experiment 2 ($h^2_R$ = 0.61) (Table 2-2).

**Table 2-2** Heritability ($h^2$) estimates and realized heritability ($h^2_R$) of late blight resistance conferred by PI 163245 calculated by parent-offspring correlation analyses and response to selection.

| | Experiment | Heritability |
|---|---|---|
| **$F_2$:$F_3$ P:O Correlation Analysis** | I | 0.76 ± 0.08 |
| | II | 0.78 ± 0.08 |
| | III | 0.81 ± 0.07 |
| | IV | 0.81 ± 0.07 |
| | Average | 0.79 ± 0.08 |
| **$F_3$:$F_4$ P:O Correlation Analysis** | I | 0.91 ± 0.05 |
| | II | 0.97 ± 0.04 |
| | Average | 0.94 ± 0.05 |
| **$F_2$:$F_4$ Realized Heritability** | I | 0.66 |
| | II | 0.61 |
| | Average | 0.64 |

## Number of resistance loci

Two methods were employed to estimate the number of resistance loci conferred by PI 163245. A total of 163 (~34%) individuals resembled the resistant parent (0-30% DS), while 167 (~30%) individuals resembled the susceptible parent (80-100% DS). Comparison of the $F_2$ individuals resembling the susceptible parent to the expected Mendelian ratio of $(¼)^n$ estimated the involvement of a single resistance locus with no dominance effects. A second method estimating the number of resistance loci used the following calculation:

$$n = (m_1 - m_2)^2/8(V_{F2} - V_{F1}) = (92.4 - 16.5)^2/8(914.6 - 299.3) = 1.17,$$

suggesting the involvement of one resistance locus and consistent with the previous estimate.

## Discussion

The *S. pimpinellifolium* accession PI 163245 exhibited strong LB resistance across all experiments and was statistically similar to LB resistant tomato accessions containing *Ph-2* or *Ph-3*, though not as resistant as NC 03220 (*Ph-2* + *Ph-3*). Additionally, the estimated $h^2$ from $F_2$:$F_3$ and $F_3$:$F_4$ experiments and the calculations of

$h^2_R$ suggested the LB resistance was highly heritable. High levels of heritability and strong LB resistance conferred by PI 163245 suggest that genetic mapping and breeding efforts are warranted.

The $F_1$ progeny were not as highly resistant as PI 163245, averaging 50.4% DS (Table 2-1). Additionally, nearly 40% of the $F_2$ population displayed intermediary levels of resistance from 30-80 % DS, suggesting the resistance is likely codominant (Fig. 2-1). Two methods estimating the number of resistance loci supported the involvement of just a single major resistance locus. However, confirmation of this conclusion requires genetic mapping.

The $h^2$ of LB resistance conferred by PI 163245 was estimated in multiple experiments and generations. P:O correlation analyses estimating $h^2$ were performed for $F_2$:$F_3$ and $F_3$:$F_4$ generations. P:O correlation analysis provides a close estimate of the narrow-sense heritability and is more reliable than estimates obtained based on variance components analysis or analysis of variance (DUDLEY AND MOLL 1969; FOOLAD AND JONES 1992; FOOLAD *et al.* 2002; MERK AND FOOLAD 2012). P:O correlation and regression analyses also avoid assumptions such as normality of distribution or similarity of environmental variances across populations and generations, which are often assumed for variance components analysis or analysis of variance (VOGEL *et al.* 1980; CASLER 1982; FOOLAD AND JONES 1992). Correlation analysis was employed in this study, rather than regression analysis, as it is more accurate when scalar differences between parental and progeny populations may have occurred due to variation in environmental conditions between experiments (FREY AND HORNER 1957; DUDLEY AND MOLL 1969; FOOLAD AND JONES 1992). P:O correlation analysis was performed over multiple generations to assess

potential effects of dominance variance on trait expression. If dominance effects are large, later filial generations are often more reliable since homozygosity is increased through additional inbreeding. Multiple experiments were conducted in each generation to reduce environmental effects and sampling error of $F_3$ and $F_4$ families.

The estimates of $h^2$ as determined by P:O correlation analyses were moderately high and similar between experiments, averaging 0.79 in $F_2$:$F_3$ generations and 0.94 in $F_3$:$F_4$ (Table 2-2). In comparison, $h^2$ estimated by $F_3$:$F_4$ correlation analysis was higher than $F_2$:$F_3$ experiments possibly due to fixation of resistance and susceptibility genes in the $F_3$ generation resulting in higher levels of covariance between parent and progeny generations. However, all $h^2$ estimates were moderately to highly heritable, suggesting that LB resistance from PI 163245 is highly transmittable via traditional breeding methods.

In order to provide a measure of the realized heritability ($h^2_R$), two rounds of selection for LB resistance were performed in the $F_2$ and $F_3$ generations. Applying the breeder's equation, $R = h^2S$, $h^2_R$ was calculated by measuring the response to selection from $F_2$ to $F_4$ generations. $F_4$ progeny were evaluated in two experiments and similar estimates were obtained, averaging 0.63. The two measures of $h^2_R$ were lower than estimates of $h^2$ obtained using P:O correlation analysis. P:O analysis can result in inflated estimates due to potential dominance effects (see equations in M&M section, pg. 44), while $h^2_R$ generally provides a better measure of the narrow-sense heritability based purely on the additive genetic variance. Additionally, measures of $h^2$ based on response to selection are often sensitive to environmental variance between generations, which could result in scalar differences (FALCONER AND MACKAY 1996). For example, if

disease pressure was higher in a later generation than when selections were initially made, the $h^2_R$ would be lower. It is expected that in more advanced filial generations (e.g. $F_6$ or later), $h^2_R$ would approach the estimates obtained using P:O correlation analyses.

All estimates of $h^2$ were moderately high, suggesting relatively simple genetic control of LB resistance in PI 163245, consistent with the two estimates of a single resistance locus. Estimates of a single resistance locus are consistent with qualitative resistance identified previously in other accessions of *S. pimpinellifolium* (BONDE AND MURPHY 1952; GALLEGLY AND MARVEL 1955; PEIRCE 1971; CHUNWONGSE *et al.* 2002; MERK AND FOOLAD 2012). This study suggests that PI 163245 could be a valuable resource for breeding LB resistance in tomato. Currently, only two LB resistance genes, *Ph-2* and *Ph-3*, are utilized commercially and both have been overcome by aggressive *P. infestans* isolates (CHUNWONGSE *et al.* 2002; FOOLAD *et al.* 2008). PI 163245 is highly resistant to several isolates of *P. infestans* from clonal lineages US-13, US-14, and US-23 (FOOLAD *et al.* 2014b). Additionally, markers often associated with *Ph-2* and *Ph-3* indicated that PI 163245 contains potentially novel LB resistance genes (FOOLAD *et al.* 2014b). Breeding efforts are currently underway to incorporate LB resistance from PI 163245 into tomato breeding material. Mapping of resistance genes and additional testing of PI 163245 LB resistance against a broad spectrum of *P. infestans* isolates and clonal lineages is necessary to determine the efficacy and durability of resistance.

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# Chapter 3 Genetic characterization and heritability of late blight resistance conferred by the *Solanum pimpinellifolium* accession PI 224710


## Abstract

Late blight (LB) is one of the most destructive diseases of tomato (*Solanum lycopersicum* L.) worldwide. Caused by the oomycete, *Phytophthora infestans* (Mont.) de Bary, the occurrence of aggressive and fungicide resistant clonal lineages has emphasized the need to identify new sources of genetic resistance to LB. In this study, the heritability ($h^2$) of LB resistance conferred by the *S. pimpinellifolium* accession PI 224710, was estimated using parent-offspring (P:O) correlation analysis in filial generations derived from a cross between PI 224710 and LB susceptible Fla.8059. Additionally, estimates of realized heritability ($h^2_R$) based on $F_2$ selections were calculated. A large $F_2$ population (n = 599) was screened for LB resistance and the most resistant (n = 71) and susceptible individuals (n = 63) were selected to develop $F_3$ progeny families. Heritability based on P:O analysis was estimated in two separate experiments and averaged 0.87. Two measures of the $h^2_R$ based on a single round of selection averaged 0.59. Two methods were employed to estimate the number of resistance loci conferred by PI 224710 and each suggested the involvement of a single major resistance locus. Based on the high $h^2$, strong LB resistance, moderate response to selection, and estimates of a single resistance locus, PI 224710 appears highly desirable for breeding LB resistance. Breeding efforts utilizing PI 224710 as a source of LB resistance are currently underway in The Pennsylvania State University's tomato breeding program.

## Introduction

Late blight (LB), caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most devastating diseases of tomato (*Solanum lycopersicum* L.) and potato (*Solanum tuberosum* L.) worldwide. LB is estimated to cause as high as 7% yield losses in tomato in the United States and similar losses are incurred worldwide (HTTP://WWW.NASS.USDA.GOV/QUICK_STATS; NOWICKI *et al.* 2012). The impact of LB on potato is even more severe, resulting in 5% yield loss in the field and 17% in storage, with estimated costs of more than $6 billion annually worldwide (GUENTHNER *et al.* 2001; HAVERKORT *et al.* 2008).

Until the late 1970s, LB was successfully managed through a combination of good cultural practices and frequent fungicide applications (FOOLAD *et al.* 2008). However, the impact of LB has substantially increased due to several factors. The first factor was the emergence of phenylamide resistant *P. infestans* clonal lineages. Clonal lineages exhibiting phenylamide resistance are of great concern to tomato and potato growers since this class of fungicides is one of the few effective systemic fungicides for treating LB infection (GISI AND COHEN 1996; GOODWIN *et al.* 1996). Fortunately in the United States, the most recent predominant clonal lineages, US-22 and US-23, are sensitive to phenylamide fungicides and consequently this class of fungicides can be used in fields infected by these lineages (FRY *et al*. 2013). The second factor was the arrival of the A2 mating type to the United States and Europe. *P. infestans* is a heterothallic organism requiring both A1 and A2 mating types to sexually reproduce and until recently, only the A1 mating type was found outside of Mexico, limiting the potential for generating new *P. infestans* lineages (FRY AND GOODWIN 1997). However, the

immigration of the A2 mating type has provided opportunities for sexual reproduction and the development of new and potentially more aggressive *P. infestans* clonal lineages (HOHL AND ISELIN 1984; DEAHL *et al.* 1991; GAVINO *et al.* 2000). In the United States, sexually derived *P. infestans* genotypes have only been detected on two occasions (GAVINO *et al.* 2000; DANIES *et al.* 2014). However, sexual populations of *P. infestans* appear to have become more common in Europe (YUEN AND ANDERSSON 2013). Furthermore, the often surprising intensity and geographic locations of LB infection, when combined with the continuously changing structure of *P. infestans* populations have highlighted the importance of the disease to both growers and researchers (FRY *et al.* 2015). An effective strategy for reducing the impact of LB is the discovery and incorporation of new sources of genetic LB resistance in tomato and potato breeding.

In potato, LB resistance has been extensively studied and more than 20 resistance genes have been cloned (JO *et al.* 2015). However, substantially fewer LB resistance genes have been identified within tomato species. In fact, only three major LB resistance genes have been used in commercial cultivars. The first LB resistance gene reported in tomato was *Ph-1*, a dominant resistance gene that only confers resistance to *P. infestans* race T-0. *Ph-1* was identified in the *S. pimpinellifolium* accessions West Virginia 19 and 731 and later mapped to the long arm of chromosome 7 (BONDE AND MURPHY 1952; GALLEGLY AND MARVEL 1955; PEIRCE 1971). While *Ph-1* was incorporated into several commercial tomato cultivars, it has no effect on the predominant *P. infestans* race T-1 and currently has limited value in tomato breeding (FOOLAD *et al.* 2008).

The second LB resistance gene, *Ph-2*, was initially identified in the *S. pimpinellifolium* accession West Virginia 700 and mapped to an 8.4 cM region on the

long arm of chromosome 10 (GALLEGLY AND MARVEL 1955; MOREAU *et al.* 1998). *Ph-2* is a codominant resistance gene that often fails in the presence of aggressive *P. infestans* isolates (GOODWIN *et al.* 1995; BLACK *et al.* 1996; FOOLAD *et al.* 2008). Additionally, *Ph-2* generally provides only a slowed rate of disease progression. However, high levels of LB resistance are obtained in cultivars containing *Ph-2* and *Ph-3* combined (GARDNER AND PANTHEE 2010b; GARDNER AND PANTHEE 2010a; PANTHEE AND GARDNER 2010).

*Ph-3* is a partially dominant gene and is widely considered the strongest LB resistance gene in tomato, conferring resistance against many *P. infestans* isolates (FOOLAD *et al.* 2008). *Ph-3* was identified in the *S. pimpinellifolium* accession L3708 (a.k.a. LA1269 and PI 265957) and is located on chromosome 9 (CHUNWONGSE *et al.* 2002). An additional minor LB resistance QTL, *qPh2.1*, was identified in L3708 and mapped to chromosome 2 (CHEN *et al.* 2014). *Ph-3* is the only cloned LB resistance gene in tomato (ZHANG *et al.* 2014) and has been transferred successfully to several tomato breeding lines and commercial hybrid cultivars (GARDNER AND PANTHEE 2010b; GARDNER AND PANTHEE 2010a; PANTHEE AND GARDNER 2010). However, certain *P. infestans* isolates have displayed virulence even against cultivars containing *Ph-3* (CHUNWONGSE *et al.* 2002; FOOLAD *et al.* 2008; R.G. GARDNER, *pers. comm.*). Consequently identifying new sources of LB resistance in tomato germplasm is necessary.

Although *Ph-1*, *Ph-2*, and *Ph-3* are the only tomato LB resistance genes to have been used commercially, additional resistance genes have been identified and mapped in *S. habrochaites*, *S. pennellii*, and additional *S. pimpinellifolium* accessions (BROUWER *et al.* 2004; BROUWER AND ST. CLAIR 2004; IRZHANSKY AND COHEN 2006; SMART *et al.*

2007; ABREU *et al.* 2008; LI *et al.* 2011; MERK *et al.* 2012; MERK AND FOOLAD 2012). Late blight resistance QTLs were identified on all 12 tomato chromosomes in the *S. habrochaites* accession LA2099 and three were fine mapped to chromosomes 4, 5, and 11 (BROUWER *et al.* 2004; BROUWER AND ST. CLAIR 2004). However, these QTLs are tightly linked with undesirable traits, including dense canopy, low yield, small fruit, and late maturity. Five QTLs were mapped in *S. habrochaites* accession LA1777, four of which co-localized with QTLs identified in LA2099 (LI *et al.* 2011). Late blight resistance has also been reported in the *S. habrochaites* accession BGH6902, though its viability in tomato breeding is limited due to low heritability and the reported involvement of as many as 28 resistance genes (ABREU *et al.* 2008). Mapping of LB resistance in the *S. pennellii* accession LA716 identified a major LB resistance QTL on chromosome 6 (SMART *et al.* 2007). However, it is unclear if this QTL provides true host resistance or is a result of its tight linkage to the self-pruning (*Sp*) locus, which may have resulted in plants outgrowing the rate of LB infection (SMART *et al.* 2007).

Several additional highly resistant *S. pimpinellifolium* accessions have been identified. Late blight resistance in the accession L3707 was reported, although mapping has not been performed (IRZHANSKY AND COHEN 2006). A screening of nearly 70 *S. pimpinellifolium* accessions identified 12 containing similar LB resistance as control lines containing *Ph-2* and *Ph-3* combined (FOOLAD *et al.* 2014b). Genetic characterization and mapping of disease resistance in one of the 12 accessions, PI 270443, estimated high heritability and LB resistance was mapped to chromosomes 1 and 10 (MERK *et al.* 2012; MERK AND FOOLAD 2012). Genetic characterization of the remaining 11 accessions is necessary to determine their viability for tomato breeding. The accession PI 224710,

exhibited late blight resistance in both field and greenhouse screenings to several *P. infestans* isolates from the clonal lineages US-13, US-14, and US-23 (FOOLAD *et al.* 2014b). In order to determine the utility of PI 224710 in tomato breeding, this study was undertaken to estimate the heritability of LB resistance and number of resistance genes.

Heritability ($h^2$) provides a measure of how well a trait corresponds between parent and progeny generations based exclusively on genetic factors. Estimation of heritability enables predictions to be made regarding the efficiency of both response to selection and potential gene mapping studies (VISSCHER *et al.* 2008). Heritability can be measured in either the broad-sense or narrow-sense. Broad-sense $h^2$ includes both additive and dominance variance, while narrow-sense $h^2$ includes only the additive variance. In most cases, the narrow-sense $h^2$ is more valuable since additive effects are most desirable for breeding.

In this study, a parent-offspring (P:O) correlation analysis approach was selected to estimate the narrow-sense $h^2$ of LB resistance conferred by PI 224710. P:O correlation and regression analyses provide a close estimate of the narrow-sense $h^2$ and in most cases are more reliable than those obtained using analysis of variance and variance component analysis (DUDLEY AND MOLL 1969; FOOLAD AND JONES 1992; FOOLAD *et al.* 2002; MERK AND FOOLAD 2012). Correlation analysis rather than regression analysis was employed in this study as it provides a more reliable estimate of $h^2$ when scalar differences could exist between parent and progeny experiments (FREY AND HORNER 1957; DUDLEY AND MOLL 1969; FOOLAD AND JONES 1992). However, if dominance genetic variance is particularly large, P:O correlation can overestimate $h^2$ in early generations. In order to provide an additional measure of heritability, the realized

heritability ($h^2_R$) based on a single round of selection was calculated. To further characterize the resistance, two methods were employed estimating the number LB resistance genes conferred by PI 224710.

## Materials and Methods

### Plant Material

Hybridizations were performed between the LB susceptible tomato breeding line Fla.8059 (pistillate parent) and the LB resistant *S. pimpinellifolium* accession PI 224710 (staminate parent). Fla.8059 has many desirable horticultural characteristics including good yield and large, ultra-firm fruit with good flavor and high lycopene content (SCOTT *et al.* 2008). However, Fla.8059 is extremely susceptible to LB. In contrast, PI 224710 is highly resistant to LB with similar levels of resistance as cultivars containing *Ph-2* and *Ph-3* combined (FOOLAD *et al.* 2014b). PI 224710 has undesirable traits including poor growth habit, dense canopy, and very small fruit.

Hybrid $F_1$ progeny were self-pollinated and a large $F_2$ population (n = 599) was developed. Highly LB resistant and susceptible plants were selected, grown to maturity, and self-pollinated to produce resistant and susceptible class $F_3$ progeny families. $F_3$ progeny families were screened for LB resistance in two separate experiments to estimate heritability based on P:O correlation analysis and determine $h^2_R$ based on response to selection (described below). In all experiments parental lines, $F_1$ progeny, and control lines were included. Controls consisted of NC 84173 (LB susceptible), New Yorker (*Ph-1*), NC 63EB (*Ph-2*), NC 870 (*Ph-3*), and NC 03220 (*Ph-2* + *Ph-3*). Seed for Fla.8059 was generously provided by John (Jay) Scott, University of Florida, Gulf Coast Research Education Center, Wimauma, FL, USA and PI 224710 seed was procured from the

USDA Plant Genetic Resource Unit (PGRU), Geneva, NY, USA. All control lines were kindly provided by Randolph Gardner, North Carolina State University, Mills River, NC, USA.

**Inoculum preparation**

The *P. infestans* isolate RS2009T1 was used for all experiments. RS2009T1 is an isolate belonging to the US-23 clonal lineage and belongs to race T-1 and mating type A1. RS2009T1 was originally collected in 2009 from a commercial tomato field in Rock Springs, PA. An isolate from the US-23 clonal lineage was selected based on its wide prevalence throughout the Northeastern United States and its virulence on tomato and potato (GUGINO AND FOOLAD 2013; FOOLAD *et al.* 2014b; GUGINO *et al.* 2014). To prepare the inoculum, the *P. infestans* isolate was cultured on LB susceptible tomato leaflets. The leaflets were placed in sterile 100 × 15 mm petri dishes containing a thin layer of 1.7% water agar inside the lid to maintain high humidity. The petri dishes were incubated for 7-11 days at 14-16 °C and 100% relative humidity on a 12 hour photoperiod. Once sufficient sporangia were produced, the inoculum was prepared by adding the infected leaflets to 500 mL of chilled water and incubating at 4 ºC for 1 hour. The solution was briefly vortexed to dislodge sporangia and facilitate zoospore release. The inoculum was filtered through cheesecloth to remove plant debris and the sporangia concentration was adjusted to 10,000 sporangia/mL using a haemocytometer and light microscope.

**$F_2$ disease evaluation**

A screening population composed of 599 $F_2$ individuals, parental lines, $F_1$ progeny, and resistant and susceptible control lines was grown in 72-cell seedling trays in

an environmentally controlled greenhouse compartment. The parental lines, $F_1$ progeny, and control lines were grown in four replications, each containing six plants and placed on opposite sides and ends of the compartment. All plants were inoculated at approximately six weeks of age. Six hours prior to inoculation, the greenhouse temperature was reduced to 16-18 ºC and high pressure foggers were used to increase the relative humidity to 95-100%. Additionally, blackout curtains were used to reduce ambient lighting for the first 24 hours to suppress the hypersensitive and salicylic acid defense responses (GRIEBEL AND ZEIER 2008; RODEN AND INGLE 2009). Clear plastic was draped around each bench to prevent direct exposure of the plants to the high pressure fog. Six hours after the high pressure fog was first initiated, the screening population was sprayed with water. Thirty minutes later, *P. infestans* inoculum was uniformly sprayed at a volume of 1 liter/1000 plants and a concentration of 10,000 sporangia/mL. A second inoculation using the same volume and concentration was conducted after 30 minutes. The following day the blackout curtains were raised to allow ambient light with no supplemental lighting.

Five days following inoculation, the plants were evaluated based on their foliar disease severity across the whole plant. Disease severity (% DS) scores were assigned to each $F_2$ individual on a scale from 0-100%. A score of 0% DS specified no late blight symptoms were observed, while a score of 100% DS indicated no remaining healthy tissue or complete defoliation. For the parental, $F_1$, and control lines % DS was assigned based on average % DS for the entire replicate. The 71 most resistant (% DS <10) and 63 most susceptible (% DS >85) surviving $F_2$ individuals were grown to maturity and self-

pollinated to produce $F_3$ progeny seed. The $F_3$ progeny families were screened for LB resistance in order to estimate the heritability of resistance.

**$F_3$ disease evaluations**

The $F_3$ progeny families were evaluated at two distinct times and are considered separate experiments (I and II). The first experiment consisted of 57 resistant and 51 susceptible $F_3$ families and the second experiment contained 61 resistant and 58 susceptible families. In most cases (83%), the $F_3$ progeny families were included in both experiments. However, insufficient seed resulted in the exclusion of 16 families from experiment I and 5 families from experiment II. The parental and control lines were included in both experiments, however due to insufficient seed, $F_1$ progeny were only included in experiment II. The experiments were conducted similarly to the $F_2$ screening experiment, however two replications totaling 10-12 individuals from each $F_3$ progeny family were evaluated in two separate close by greenhouse sections to accommodate the large population size. Experiment I was evaluated six days after inoculation and experiment II was evaluated after five days. Each $F_3$ individual was assigned a % DS score and the average was calculated for all $F_3$ progeny families. The average % DS was determined for the parental, $F_1$, and control lines as described previously.

**Data analysis**

The heritability ($h^2$) of LB resistance was determined based on the % DS of the selected $F_2$ individuals and the means of their respective $F_3$ progeny families. The parent-offspring (P:O) correlation analysis was performed as follows:

$$h^2(\mathrm{F}_{2:3}) = r_{\mathrm{F2:F3}} = \frac{Cov_{F3,F2}}{(V_{F3}V_{F2})^{1/2}} = \frac{V_A + 1/2\,V_D}{[(V_A + 1/4\,V_D + 1/n\,V_E)(V_A + V_D + V_E)]^{1/2}},$$

where *r* is the correlation coefficient, *Cov* is the covariance of the $F_2$ and $F_3$ generations, $V_{F2}$ is the variance of the selected individuals from the $F_2$ generation, $V_{\overline{F3}}$ is the variance of the $F_3$ progeny family means, $V_A$, $V_D$, and $V_E$ are the additive, dominance, and environmental variances, respectively, and *n* is the number of individuals in each $F_3$ progeny family (FALCONER AND MACKAY 1996). The standard errors of the heritabilities were estimated by the equation $h^2(F_{2:3}) = [(1-r^2_{F2:F3})/(n-2)]^{1/2}$, where *n* is the number of $F_3$ families.

The realized heritability ($h^2_R$) was measured based on response to selection and using the breeder's equation:

$$h^2_R = \frac{R_{F2:F3}}{S_{F2:F3}},$$

where *R* is the response to selection and *S* is the selection differential. *R* was determined by the change in mean % DS between the $F_2$ population and the resistant class $F_3$ progeny. The selection differential, *S*, was calculated by the difference between the means of selected resistant individuals and resistant class $F_3$ progeny families (FALCONER AND MACKAY 1996).

To estimate the number of loci contributing to LB resistance, two methods were employed. The first estimate compared the proportion of LB susceptible $F_2$ individuals, with similar % DS as the homozygous susceptible parent, to the expected Mendelian ratio $(¼)^n$, where *n* is the minimum number of resistance loci. The second method estimated the number of resistance loci utilizing the equation:

$$n = \frac{(m_1 - m_2)^2}{8(V_{F2} - V_{F1})},$$

where $n$ is the number of resistance loci, $m_1$ and $m_2$ are the mean % DS of the parental lines (Fla.8059 and PI 224710) and $V_{F2}$ and $V_{F1}$ are the variances of the $F_2$ and $F_1$ generations (WRIGHT 1952; FALCONER AND MACKAY 1996).

Mean comparisons of the parental, $F_1$, and resistant and susceptible control lines were calculated using Tukey's HSD test.

## Results

### Disease response of parental, $F_1$, and control lines

The % DS of parental lines, $F_1$, $F_2$, $F_3$, and controls are displayed in Table 3-1. The LB resistance of PI 224710 was high in all experiments, averaging 13.9% DS. In no experiment did the average % DS of any replicate exceed 25%. PI 224710 was not statistically different from LB resistant control lines NC 870 (*Ph-3*) and NC 03220 (*Ph-2* + *Ph-3*), averaging 13.8% and 3.4% DS respectively. PI 224710 outperformed NC 63EB (*Ph-2*), which exhibited nearly twice the average % DS of PI 224710. In contrast, Fla.8059 was extremely susceptible to LB across all experiments, averaging close to 90% DS. Fla.8059 was not statistically different from susceptible controls NC 84173 and New Yorker (*Ph-1*). The performance of $F_1$ progeny was not statistically different from PI 224710, suggesting the resistance may be under dominant control.

**Table 3-1** Late blight disease severity (% defoliation/foliar disease symptoms ± SD) for resistant (PI 224710) and susceptible (Fla. 8059) parents, $F_1$ progeny, control lines, and $F_2$ and $F_3$ experimental populations.

| | Genotype | Number of Individuals or Families | % DS[1] | Range (% DS) |
|---|---|---|---|---|
| | $P_1$ (Fla.8059) | 120 | 89.4 ± 11.2[a] | 60-100 |
| | $P_2$ (PI 224710) | 120 | 13.9 ± 5.9[bc] | 5-25 |
| | $F_1$ | 48 | 17.1 ± 6.4[cd] | 7-25 |
| | NC 84173 | 96 | 95.4 ± 5.1[a] | 80-100 |
| | New Yorker (*Ph-1*) | 120 | 94.1 ± 5.0[a] | 80-100 |
| | NC 63EB (*Ph-2*) | 120 | 26.7 ± 19.3[d] | 5-70 |
| | NC 870 (*Ph-3*) | 120 | 13.8 ± 7.1[bc] | 5-25 |
| | NC 03220 (*Ph-2* + *Ph-3*) | 96 | 3.4 ± 1.7[b] | 0-7 |
| | $F_2$ population | 599 | 52.5 ± 32.1 | 0-100 |
| **Experiment I** | $F_2$ selected individuals (resistant class) | 57 | 3.9 ± 2.7 | 0-10 |
| | $F_2$ selected individuals (susceptible class) | 51 | 91.6 ± 3.3 | 85-97 |
| | $F_3$ progeny families (resistant class) | 57 | 25.1 ± 29.3 | 0-100 |
| | $F_3$ progeny families (susceptible class) | 51 | 80.3 ± 24.3 | 2-100 |
| **Experiment II** | $F_2$ selected individuals (resistant class) | 61 | 4.0 ± 2.6 | 0-10 |
| | $F_2$ selected individuals (susceptible class) | 58 | 91.7 ± 3.3 | 85-97 |
| | $F_3$ progeny families (resistant class) | 61 | 17.2 ± 26.9 | 0-98 |
| | $F_3$ progeny families (susceptible class) | 58 | 83.0 ± 24.8 | 5-100 |

[1]Mean comparisons of parental and control lines were determined using Tukey's HSD test ($P \leq 0.05$) and are denoted by superscript (a-d).

## $F_2$ disease response

The $F_2$ population (n = 599) ranged from 0-100% DS and averaged 52.5% which was similar to the mid-parent value of 51.7% DS (Fig. 3-1). The $F_2$ population generally resembled a bimodal distribution. Slightly fewer than half (47%) the $F_2$ individuals displayed ≤50% DS, although the population was somewhat skewed towards both susceptibility and resistance. Approximately 61% of the population fell within the upper and lower quartiles of the phenotypic distribution. In total, 170 (28%) plants displayed similar levels of LB resistance as PI 224710 (<25% DS), while 140 (23%) $F_2$ individuals were similar to the susceptible parent (>80% DS). The population was non-normally distributed ($P < 0.001$) based on the Shapiro-Wilks test for normality.

The most resistant and susceptible individuals (n = 108) were retained from the $F_2$ population for development of $F_3$ progeny families. The selected resistant class $F_2$ individuals (n = 57) averaged 3.9% DS and ranged from 0-10% DS, while the selected LB susceptible individuals (n = 51) averaged 91.6%, ranging from 85-97% DS (Table 3-1, Figs. 2-2, 2-3). The % DS of selected $F_2$ individuals was generally similar to the resistant or susceptible parent (Table 3-1). However, the average % DS of PI 224710 was slightly higher than the average of the selected resistant individuals, suggesting the resistant $F_2$ individuals were either under lower disease pressure or that additional genetic factors from the susceptible parent were contributing to higher levels of LB resistance.

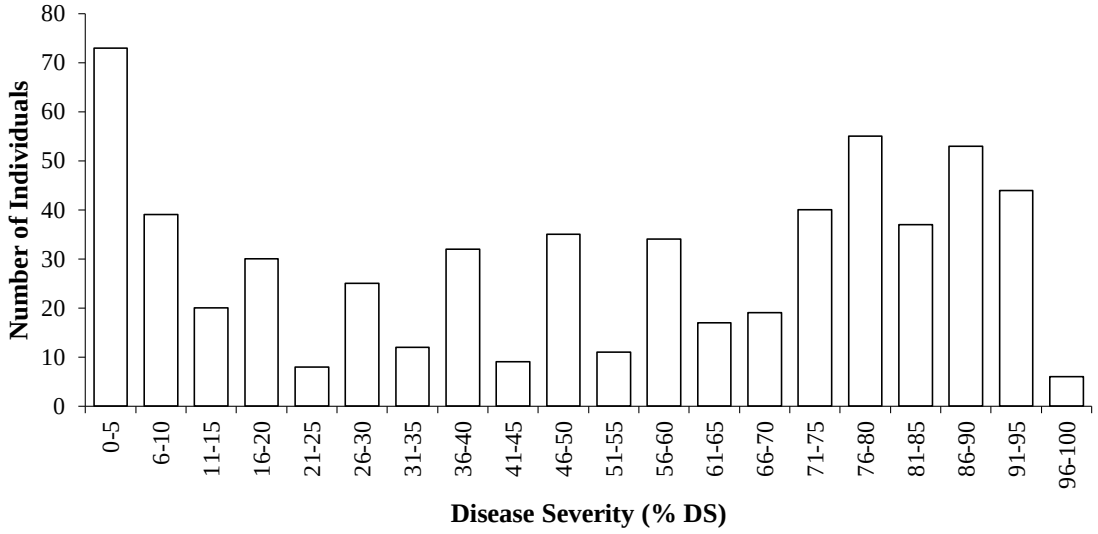


**Figure 3-1** Frequency distribution of disease severity (% DS) of the $F_2$ population (n = 599). Foliar % DS was measured as a percentage of whole plant foliar disease symptoms/defoliation.

## $F_2$:$F_3$ parent-offspring correlation analysis

The $F_3$ progeny families were screened two times and are considered separate experiments. Each experiment contained two replications for each $F_3$ progeny family. Correlation between replicates was high for both experiments ($r \geq 0.8$, $P < 0.001$) so replicates were pooled for estimating $h^2$.

In most cases, the resistant and susceptible class $F_3$ progeny families resembled their respective $F_2$ parents (Figs. 3-2, 3-3). In experiment I, the average % DS of the resistant class $F_3$ progeny families (n = 57) averaged 25.1%, which was somewhat higher than the resistant class $F_2$ parents and PI 224710 (Table 3-1). The average % DS of the susceptible $F_3$ progeny families (n = 51) was 80.3%, slightly lower than the selected susceptible $F_2$ parents (91.6% DS). Four of the resistant class $F_3$ progeny families were somewhat susceptible to LB, averaging >60% DS and nine of the susceptible class $F_3$ progeny families were more resistant than expected on average (% DS <60) (Fig. 3-2). Despite these outliers, in experiment I the $h^2$ estimated by P:O correlation was 0.85, suggesting LB resistance conferred by PI 224710 was highly heritable (Table 3-2).

To confirm the heritability of resistance, a second experiment was conducted which consisted of 61 resistant class and 58 susceptible class $F_3$ progeny families. In experiment II, the mean % DS of the resistant class $F_3$ progeny families was 17.2%, which was somewhat higher than their resistant $F_2$ parents, though similar to the resistant parent, PI 224710. The average % DS (83.0%) of the susceptible class $F_3$ progeny families, was slightly below the mean of the susceptible class $F_2$ parents. Two of the resistant class $F_3$ families were more susceptible to LB than expected (% DS >60) and seven susceptible class $F_3$ families were more resistant than expected (% DS <60) (Fig. 3-3). However, the estimate of $h^2$ in experiment II was 0.88, fairly consistent with experiment I (Table 3-2).

The two estimates of heritability obtained from experiments I and II were highly similar, demonstrating consistency and repeatability of experiments. Furthermore, the anomalous $F_3$ progeny families averaging higher or lower % DS than their respective $F_2$

parents, were generally the same in each experiment, suggesting the $F_2$ parents were potentially heterozygous at resistance loci or scored incorrectly due to variation in disease pressure.

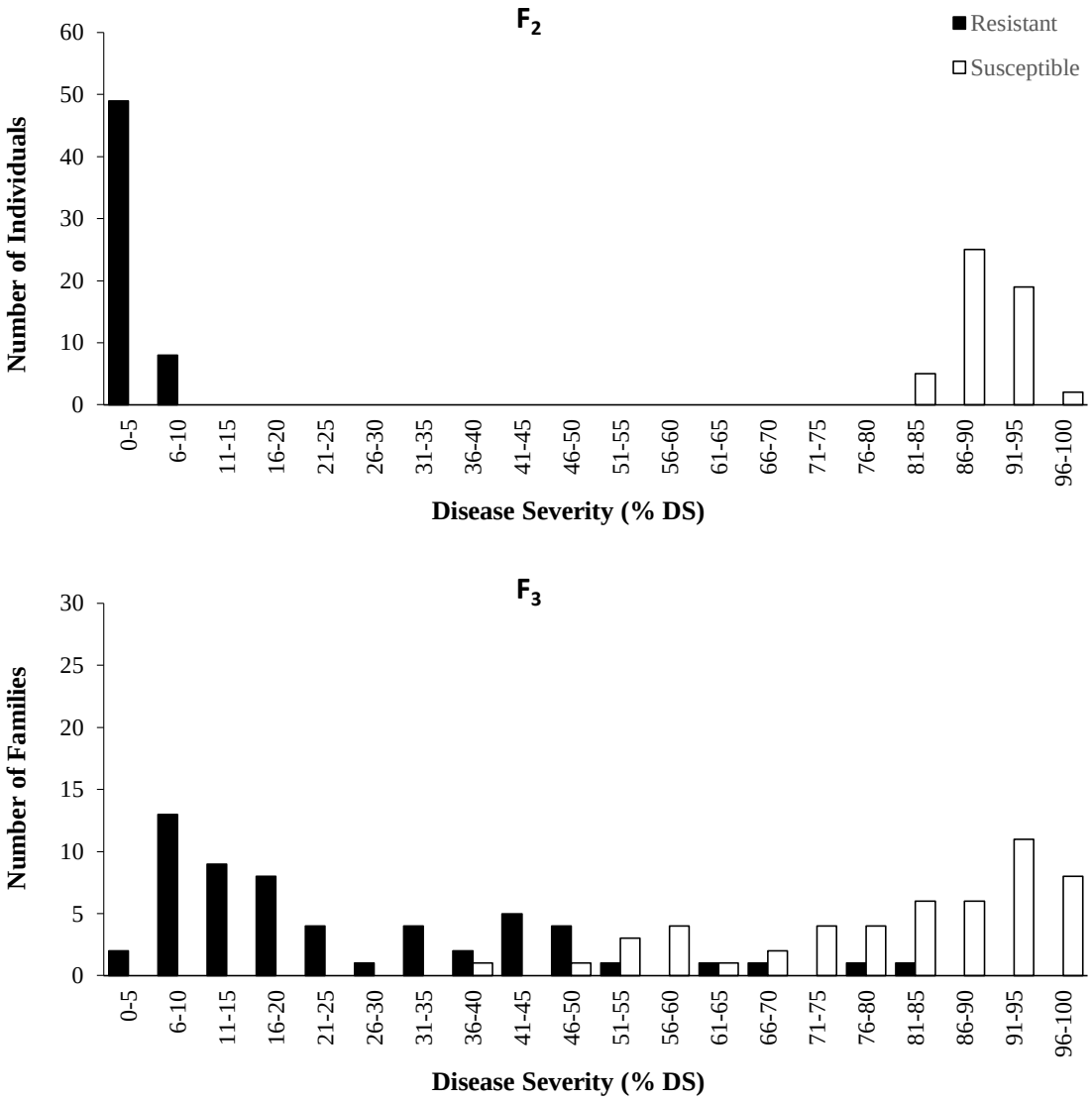


**Figure 3-2** Experiment I frequency distributions of disease severity (% DS) for selected resistant and susceptible $F_2$ individuals (top) and mean % DS of $F_3$ progeny families (bottom). Foliar % DS was measured as a percentage of whole plant foliar disease symptoms/defoliation.

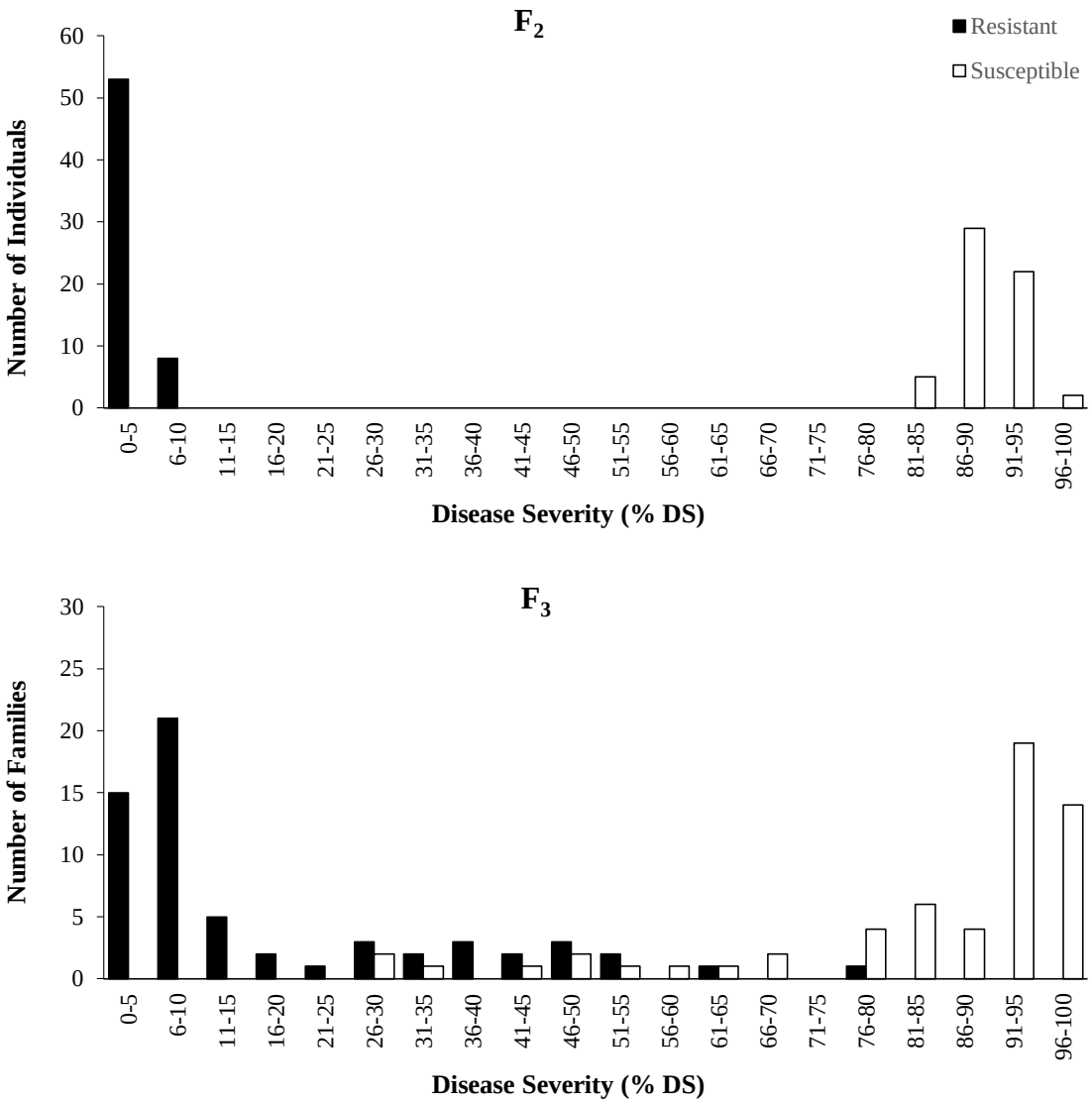


**Figure 3-3** Experiment II frequency distributions of disease severity (% DS) for selected resistant and susceptible $F_2$ individuals (top) and mean % DS of $F_3$ progeny families (bottom). Foliar % DS was measured as a percentage of whole foliar disease symptoms/defoliation.

## Realized heritability

In addition to estimates of $h^2$ obtained via P:O correlation analyses, the realized heritability ($h^2_R$) was determined based on a single round of selection for LB resistance in the $F_2$ generation. Two $F_3$ experiments were performed and thus $h^2_R$ was calculated twice. In experiment I, the $h^2_R$ based on 57 resistant class $F_3$ progeny families was 0.48, lower than $h^2$ estimates calculated by P:O correlation (Table 3-2). However, $h^2_R$ was 0.69 in

experiment II (n = 61), more similar than the first experiment to estimates obtained based on $F_2$:$F_3$ correlation analysis. The average $h^2_R$ based on the two experiments was 0.59.

**Table 3-2** Estimates of heritability ($h^2$) and realized heritability ($h^2_R$) of late blight resistance conferred by PI 224710 determined by parent-offspring correlation analyses and response to selection.

| | Experiment | Heritability |
|---|---|---|
| **$F_2$:$F_3$ P:O Correlation Analysis** | I | 0.85 ± 0.05 |
| | II | 0.88 ± 0.04 |
| | Average | 0.87 ± 0.05 |
| **$F_2$:$F_3$ Realized Heritability** | I | 0.48 |
| | II | 0.69 |
| | Average | 0.59 |

## Number of resistance loci

The number of loci involved in LB resistance conferred by PI 224710 was estimated using two methods. Method one compared the number of $F_2$ individuals with similar LB susceptibility as Fla.8059 to the expected Mendelian ratio. In total, 140 individuals (23%) displayed >80% DS, estimating the involvement of a single resistance locus with no dominance effects. However, the dearth of dominance effects suggested by the $F_2$ distribution conflicts with the high levels of LB resistance observed in the $F_1$ progeny (discussed below).

The second method of estimating the minimum number of LB resistance loci was calculated as follows:

$n = (m_1 - m_2)^2/[8(V_{F2} - V_{F1}) = (89.4\text{-}13.9)^2/[8(1027.9 - 36.1)] = 0.72,$

which is consistent with the previous estimate of just one major LB resistance locus.

## Discussion

The *S. pimpinellifolium* accession PI 224710 was highly resistant to LB in all experiments (Table 3-1). PI 224710 was not statistically different from control lines NC 63EB (*Ph-2*), NC 870 (*Ph-3*), or NC 03220 (*Ph-2* + *Ph-3*), demonstrating the LB

resistance conferred by this accession is as good as or better than previous LB resistance genes utilized in tomato breeding. Additionally, PI 224710 was previously screened in several field and greenhouse experiments and was found highly resistant to several *P. infestans* isolates (FOOLAD *et al.* 2014b). In fact, PI 224710 outperformed L3708, the source of *Ph*-3 (FOOLAD *et al.* 2014b, FOOLAD *et al*., unpublished data). The combination of these factors demonstrates the potential of PI 224710 for breeding tomato LB resistance.

The average % DS of the $F_1$ generation was not statistically different from that of the resistant parent (Table 3-1), suggesting LB resistance in PI 224710 is under dominant control. However, the distribution of the $F_2$ population suggests that the LB resistance is more complex than a single dominant resistance locus (Fig. 3-1). Genetic factors explaining the $F_2$ distribution could include additive or partial dominance effects or the presence of multiple resistance loci with additive, dominance, or epistatic effects. Environmental effects may also have influenced the $F_2$ % DS distribution. However, the high heritability of LB resistance and nearly bimodal distribution of the $F_2$ population support the presence of one major resistance locus with partial dominance effects. This assertion is also supported by two estimates suggesting the involvement of a single resistance locus. In order to confirm this hypothesis, mapping of the resistance genes affecting LB resistance in PI 224710 was necessary (Chapter 5).

The $h^2$ of LB resistance conferred by PI 224710 was estimated using P:O correlation analysis. Parent-offspring correlation provides a close estimate of the narrow-sense heritability and is more reliable than estimates obtained from analysis of variance or variance components analysis (DUDLEY AND MOLL 1969; FOOLAD AND JONES 1992;

FOOLAD *et al.* 2002; MERK AND FOOLAD 2012). Correlation and regression analyses avoid assumptions commonly made in analysis of variance and variance components analysis such as normal population distribution and similarity of environmental variances between experiments (VOGEL *et al.* 1980; CASLER 1982; FOOLAD AND JONES 1992). Furthermore, correlation analysis is generally better than regression analysis as it provides better accuracy when estimating $h^2$ if scalar differences in environmental conditions could exist between parent and progeny generations (FREY AND HORNER 1957; DUDLEY AND MOLL 1969; FOOLAD AND JONES 1992). To avoid the effects of environmental variation or variation caused by sampling of $F_3$ families, two replicated $F_3$ experiments were conducted. In each experiment, the $F_3$ progeny families generally resembled the disease phenotype of their $F_2$ parent (Figs. 3-2, 3-3). In addition, the average % DS of $F_3$ families were similar in both experiments, supporting their reliability and accuracy. Consequently, these $h^2$ estimates obtained by P:O correlation analysis are likely a good representation of the expected response to artificial selection.

In both P:O correlation experiments the $h^2$ estimates were similar and high, averaging 0.87 (Table 3-2). The high $h^2$ and degree of LB resistance observed in PI 224710 suggest that the resistance could be utilized with high probability of success in tomato breeding. This does not discount the potential for issues such as linkage drag which has previously been reported for LB resistance conferred by *S. habrochaites* (BROUWER AND ST. CLAIR 2004). However, S. *pimpinellifolium* is more closely related to the cultivated tomato and has been used extensively with good success in tomato breeding. Additionally, preliminary backcross breeding efforts have been largely successful. The high estimates of $h^2$ were also supported by a moderate estimate of $h^2_R$

($h^2_R$ = 0.59) calculated based on a single round of selection. It is expected that $h^2_R$ would increase and approach the P:O correlation estimates of $h^2$ with additional rounds of selection. However, P:O correlation can sometimes result in inflated estimates of the narrow-sense heritability based on potential dominance effects, while $h^2_R$ can provide a more accurate measurement. In contrast, estimates of $h^2_R$, especially when based on a single round of selection, are sensitive to environmental variances between generations, resulting in scalar differences (FALCONER AND MACKAY 1996). Despite differences between the two $h^2$ values, both methods indicate the LB resistance conferred by PI 224710 is heritable.

Since *Ph-2* and *Ph-3* are the only widely utilized LB resistance genes in tomato breeding, identifying and incorporating new sources of genetic LB resistance is critical. The importance of discovering new sources of LB resistance is emphasized by the development of fungicide resistant *P. infestans* clonal lineages and the reported resistance breakdown of cultivars containing either *Ph-2* or *Ph-3* (CHUNWONGSE *et al.* 2002; FOOLAD *et al.* 2008). PI 224710 was highly resistant to LB in field, greenhouse and detached leaflet studies against multiple *P. infestans* isolates from three clonal lineages (FOOLAD *et al.* 2014a; FOOLAD *et al.* 2014b). Additionally, estimates of $h^2$ obtained in this study suggest LB resistance conferred by PI 224710 is highly heritable. However, in order to determine if the resistance conferred by PI 224710 is unique or corresponds to previously reported resistances genes, gene mapping studies are required.

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# Chapter 4 Mapping of late blight resistance in the wild tomato accession PI 163245


## Abstract

Late blight (LB), caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most devastating diseases of tomato worldwide. Until recently, LB was controlled primarily through heavy fungicide applications and good cultural practices. However, the threat of LB has grown due to the emergence of new aggressive and fungicide resistant *P. infestans* clonal lineages. Consequently, identifying genetic resistance to LB has become a priority in tomato breeding. Previously, the *S. pimpinellifolium* accession PI 163245 was identified as a promising source of LB resistance in tomato. In order to identify genomic loci associated with LB resistance in this accession, an $F_2$ mapping population (n = 560) derived from a cross between a LB susceptible tomato breeding line (Fla. 8059) and PI 163245 was developed. A trait-based analysis (TBA) approach (a.k.a selective genotyping) was employed to identify and map LB resistance loci in PI 163245. SNPs were discovered between the two parents by sequencing genomic reduced representation libraries (RRLs). Comparison of the two sequences yielded 33,385 putative SNPs. The $F_2$ mapping population was screened for LB resistance, and the most resistant (n = 39) and susceptible (n = 35) phenotypic classes were selected and genotyped with 233 SNP markers distributed throughout the genome. The QTL mapping analysis indicated that four genomic regions on chromosomes 2, 3, 10 and 11 were associated with LB resistance conferred by PI 163245. Breeding efforts and development of near-isogenic lines (NILs) for fine mapping are currently in progress.

## Introduction

The cultivated tomato, *S. lycopersicum* L., is one of the most important crops worldwide. Tomato is the most economically valuable vegetable crop, estimated at nearly $60 billion annually, and among vegetables is second only to potato in volume consumed per capita (FAOSTAT3.FAO.ORG). Additionally, tomato has high nutritional importance in many diets. For example, it is the number one dietary source of vitamins, minerals, and phenolic antioxidants in the United States (RICK 1980; NGUYEN AND SCHWARTZ 1999). Tomato's economic and dietary contributions emphasize the importance of breeding for improved yield, nutrition, and biotic and abiotic stress resistance. However, it is estimated that the cultivated tomato contains as little as 5% of the total genetic variation found within all tomato species (MILLER AND TANKSLEY 1990). Consequently, wild tomato species are an important resource for cultivar improvement.

While *S. lycopersicum* has a narrow genetic base, a large number of genetic resources are available to tomato breeders. More than 80,000 cultivated and wild tomato accessions are stored in seedbanks worldwide (BAUCHET AND CAUSSE 2012). Furthermore, there are 13 species of tomato, several of which are useful for tomato breeding. The species *S. pimpinellifolium*, *S. peruvianum*, and *S. habrochaites* have been utilized most frequently, especially with regards to improvement of disease resistance (FOOLAD 2007). Worldwide, tomatoes are susceptible to more than 200 diseases including viruses, bacteria, nematodes, and fungi depending on the geographic region (LUKYANENKO 1991).

One of the most important tomato diseases is late blight (LB), caused by the oomycete *Phytophthora infestans* (Mont.) de Bary and most notable for its role in the

Irish Potato Famine. Late blight has been reported to affect several species within the Solanaceae family, although its most common hosts are tomato and potato species (BECKTELL *et al.* 2006). In potato, LB results in more than $6 billion in economic losses from reduced yields and the costs associated with disease management (HAVERKORT *et al.* 2008; HAVERKORT *et al.* 2009). In tomato, as much as 7% of tomato yields are lost annually in the United States due to LB (NOWICKI *et al.* 2012).

*P. infestans* spreads most rapidly in cool and humid conditions, causing dark brown, purple, or black lesions that can develop on all above ground portions of the plant as well as potato tubers. *P. infestans* is a heterothallic organism, requiring both A1 and A2 mating types for sexual reproduction, and is composed of two known races: T-0 and T-1. Previously, LB was controlled primarily through frequent fungicide applications and participation in good cultural practices such as fallow and crop rotation of potato fields and timely destruction of infected plant material (FOOLAD *et al.* 2008). However, in the 1980s, the A2 mating type was identified in Europe and soon after in the United States, placing renewed emphasis on the disease (HOHL AND ISELIN 1984; DEAHL *et al.* 1991). The existence of both A1 and A2 mating types within Europe and the United States has increased potential opportunities for sexual reproduction and the generation of new and potentially more aggressive clonal lineages. Fortunately, sexual populations of *P. infestans* have been rare so far within the United States (GAVINO *et al.* 2000; DANIES *et al.* 2014) though they are becoming more frequent in Europe (YUEN AND ANDERSSON 2013). Additionally, several new strains of *P. infestans* have developed resistance to phenylamides, one of the most effective classes of systemic fungicides (GISI AND COHEN

1996; GOODWIN *et al.* 1996). Consequently, discovery and incorporation of new genetic resistance to LB has become a priority in potato and tomato breeding.

Late blight resistance in potato has been extensively studied and more than 20 LB resistance genes have been cloned (JO *et al.* 2015). All cloned resistance genes encode CC-NBS-LRR proteins and it is believed the majority of *R* genes associated with LB resistance encode proteins belonging to this class (VLEESHOUWERS *et al.* 2011). Late blight resistance in potato has been identified in at least 10 potato species on all 12 chromosomes, most notably in the wild species *S. demissum* and *S. bulbocastanum* (BALLVORA *et al.* 2002; SONG *et al.* 2003; VAN DER VOSSEN *et al.* 2003; HUANG *et al.* 2005; VOSSEN *et al.* 2005; LOKOSSOU *et al.* 2009; LI *et al.* 2011a). In comparison, relatively few LB resistance genes have been located in tomato. However, recently research with the intent of identifying new sources of LB resistance in tomato has increased.

Several LB resistance genes have been identified in *S. pimpinellifolium*. The first tomato LB resistance gene discovered was *Ph-1*, a dominant gene located on the long arm of chromosome 7 (BONDE AND MURPHY 1952; GALLEGLY AND MARVEL 1955; PEIRCE 1971). *Ph-1* was originally discovered in accessions West Virginia 19 and 731 (BONDE AND MURPHY 1952). While *Ph-1* was implemented in several tomato cultivars such as Nova and New Yorker, it is ineffective against the predominant *P. infestans* race T-1 (PEIRCE 1971). Consequently, *Ph-1* is currently considered of little value in tomato breeding (FOOLAD *et al.* 2008). The second tomato LB resistance gene, *Ph-2* was identified in the accession West Virginia 700 (GALLEGLY AND MARVEL 1955). *Ph-2* confers incompletely dominant resistance and was mapped to the distal portion of the

long arm of chromosome 10 (MOREAU *et al.* 1998). While *Ph-2* is effective against *P. infestans* race T-1, it is overcome by more aggressive *P. infestans* isolates and only reduces the rate of disease progression (GOODWIN *et al.* 1995; BLACK *et al.* 1996; FOOLAD *et al.* 2008). However, *Ph-2* is currently one of the more useful sources of LB resistance in tomato breeding especially when combined with other LB resistance genes such as *Ph-3* (GARDNER AND PANTHEE 2010b; GARDNER AND PANTHEE 2010a; PANTHEE AND GARDNER 2010). *Ph-3* is currently the best source of single-gene resistance in tomato. Discovered in the accession L3708 (a.k.a. LA1269 and PI365957), *Ph-3* is a partially dominant gene conferring a high level of LB resistance against a wide range of *P. infestans* isolates. *Ph-3* was mapped to the long arm of chromosome 9 (CHUNWONGSE *et al.* 2002) and is the only LB resistance gene that has been cloned in tomato (ZHANG *et al.* 2014). Similarly to all previously cloned potato LB resistance genes, *Ph-3* encodes a CC-NBS-LRR class protein. However, even *Ph-3* has been overcome by certain *P. infestans* isolates (CHUNWONGSE *et al.* 2002; FOOLAD *et al.* 2008). Additionally, a minor LB resistance QTL was reported in L3708 and mapped to the short arm of chromosome 2 (CHEN *et al.* 2014). A promising source of LB resistance, *Ph*-5, was mapped in the *S. pimpinellifolium* accession, PI 270443 (MERK *et al.* 2012). PI 270443 is highly resistant to LB, providing similar resistance as control lines containing *Ph-2* and *Ph-3* combined. *Ph-5* was highly heritable and was mapped to regions on chromosomes 1 and 10 (MERK *et al.* 2012; MERK AND FOOLAD 2012).

Late blight resistance was also mapped in wild tomato species *S. habrochaites* and *S. pennellii*. Late blight resistance QTLs on all 12 tomato chromosomes were reported in the *S. habrochaites* accession LA 2099 (BROUWER *et al.* 2004). Three of the

genes with the strongest phenotypic effects were fine mapped to chromosomes 4, 5, and 11 (BROUWER AND ST. CLAIR 2004). Mapping of LB resistance in the *S. habrochaites* accession LA 1777 identified five resistance QTLs, four of which co-localized with resistance previously identified in LA 2099, although one novel QTL was found on chromosome 4 (LI *et al.* 2011b). However, many undesirable horticultural characteristics are tightly linked with these LB resistance QTLs, which have prevented their incorporation into commercial tomato cultivars (BROUWER AND ST. CLAIR 2004). Late blight resistance has also been mapped in the *S. pennellii* accession LA 716, and a QTL of moderate phenotypic effect was discovered on chromosome 6 (SMART *et al.* 2007). However, this QTL was tightly linked to the self-pruning (*Sp*) locus and it is unclear how indeterminate growth habit affected the reported resistance.

Previously, several *S. pimpinellifolium* accessions were identified with high levels of LB resistance statistically similar to that of *Ph-2* and *Ph-3* combined (FOOLAD *et al.* 2014). The accession PI 163245 was selected for further genetic characterization based on its high levels of LB resistance against multiple clonal lineages in both field and greenhouse experiments. Parent-offspring (P:O) correlation analysis based on $F_2$:$F_3$ and $F_3$:$F_4$ generations indicated the LB resistance was highly heritable, averaging 0.79 and 0.94 respectively. The realized heritability ($h^2_R$) was also estimated based on $F_2$:$F_4$ selection response and averaged 0.64, indicating that resistance was highly heritable and potentially useful for breeding (discussed in Chapter 2). Consequently, genetic mapping efforts were initiated.

Genetic mapping identifies the relationship between an organism's genotype and phenotype. Determining this relationship can greatly increase the speed of selective

breeding by facilitating marker-assisted selection (MAS) or transgenesis. Two approaches can be used for conducting linkage mapping. The first approach is marker-based analysis (MBA), which consists of genotyping and phenotyping an entire mapping population for one or more traits (THODAY 1961). The advantage of this method is that it allows for simultaneous mapping of multiple traits if the population was phenotyped for more than one trait. However, when only a single trait is studied, MBA may be less desirable due to higher genotyping costs.

In the second mapping approach, trait-based analysis (TBA; a.k.a selective genotyping), only individuals within the tails of the phenotypic distribution are genotyped (STUBER *et al.* 1980; LEBOWITZ *et al.* 1987; LANDER AND BOTSTEIN 1989). This approach can be more desirable than MBA when only a portion of the mapping population survives or if genotyping costs are substantially higher than the costs associated with raising and scoring individuals for the trait (LEBOWITZ *et al.* 1987). TBA has equivalent power to MBA when examining a single trait as long as a sufficiently large mapping population is phenotyped (DARVASI AND SOLLER 1992). Additionally, TBA can be particularly useful when attempting to identify QTLs with large phenotypic effects, which are generally more desirable for breeding purposes (NAVABI *et al.* 2009). Unidirectional (genotyping of one phenotypic class) or bidirectional (genotyping of both phenotypic classes) TBA can be conducted. However, bidirectional is at least as powerful as unidirectional analysis and has the advantage of diminishing effects of skewed segregation caused by factors other than selections for the desired trait (NAVABI *et al.* 2009). Since LB resistance is the only trait targeted in this study, a TBA approach was selected.

The process of gene/QTL mapping requires a large number of polymorphic genetic markers. SNPs are rapidly becoming the marker of choice for most genetic mapping studies due to their abundance and the diminishing costs of genotyping. The availability of complete reference genome sequences for many crop species, including tomato, has made genomic analyses, such as genome assembly, much less demanding. Combined with the falling costs of next-generation sequencing (NGS), it is possible to rapidly discover many SNPs spanning the entire genome. A relatively simple and cost effective method for SNP discovery is the construction and sequencing of reduced representation libraries (RRLs).

Using RRLs substantially decreases genome complexity. Excluding the redundant and non-informative repetitive regions of the genome from sequencing allows more re-sampling and increases both the coverage at informative genomic regions and the accuracy of SNP calling (ALTSHULER *et al.* 2000). This method of SNP identification has been successfully used for human (ALTSHULER *et al.* 2000), soybean (HYTEN *et al.* 2010), sorghum (NELSON *et al.* 2011), and flax (KUMAR *et al.* 2012).

The objectives of this study were to construct a genetic map and identify the genomic regions associated with LB resistance conferred by the *S. pimpinellifolium* accession PI 163245. Selective genotyping (TBA) was performed in an $F_2$ mapping population derived from a LB susceptible tomato breeding line (Fla. 8059) and the LB resistant wild accession PI 163245. SNPs were identified between the two parents through sequencing of RRLs, and bidirectional selective genotyping was performed to identify QTLs contributing to LB resistance in PI 163245.

## Materials and Methods

### Plant Material

Hybridizations were performed between the LB resistant accession PI 163245 and LB susceptible breeding line Fla. 8059 (pistillate parent). PI 163245 is a *S. pimpinellifolium* accession with strong LB resistance in field and greenhouse conditions, with indeterminate growth habit, exerted stigmas, and yellow fruit. The LB susceptible tomato breeding line selected for this study, Fla. 8059, is an inbred line previously used in development of commercial $F_1$ hybrid cultivars. Fla. 8059 has large firm fruit, high lycopene content, and good flavor (SCOTT *et al*. 2008). The $F_1$ progeny were grown to maturity and self-pollinated to produce $F_2$ seed. A large $F_2$ population (n = 560) was grown and evaluated for LB resistance in a controlled greenhouse and the most resistant and susceptible individuals were identified and retained for production of $F_3$ progeny and confirmation of resistance or susceptibility. Subsequently, the confirmed resistant and susceptible parental $F_2$ individuals were selected for genotyping (described below). Each LB disease screening contained parental lines, $F_1$ progeny, and LB resistant and susceptible control genotypes including NC 84173 (LB susceptible), New Yorker (*Ph-1*), NC 63EB (*Ph-2*), NC 870 (*Ph-3*), and NC 03220 (*Ph-2* + *Ph-3*). Original seed of PI 163245 was received from the USDA Plant Genetics Resources Unit (PGRU), Geneva, NY, USA and Fla. 8059 seed was provided by John (Jay) Scott at the University of Florida, Gulf Coast Research & Education Center, Wimauma, FL, USA. Seed for all control lines was provided by R.G. Gardner at the North Carolina State University, Mills River, NC, USA.

**Inoculum preparation**

The *P. infestans* isolate RS2009T1 was used for all experiments. RS2009T1 is an aggressive isolate belonging to the clonal lineage US-23, which is widely prevalent throughout the Northeastern United States. (GUGINO AND FOOLAD 2013; FOOLAD *et al.* 2014; GUGINO *et al.* 2014). RS2009T1 was collected from a commercial tomato field in Rock Springs, PA in 2009 and is race T-1 and mating type A1. The isolate was cultured on LB susceptible tomato leaflets in 100 × 15 mm Petri dishes. Each Petri dish contained a thin layer of 1.7% water agar on the inside of the lid to maintain high humidity. The infected leaflets were incubated at 14-16 ºC and 100% relative humidity (RH) on a 12 hour photoperiod for 7-11 days to promote abundant sporangia production. Inoculum was prepared by placing infected leaflets in 4 ºC distilled water for one hour. The inoculum was then vortexed briefly to dislodge sporangia and the suspension was filtered through cheesecloth to eliminate leaf debris. Using a haemocytometer and light microscope, the sporangia concentration was adjusted to 10,000 sporangia/mL.

**$F_2$ and $F_3$ disease evaluations and selections**

An $F_2$ population consisting of 560 individuals was screened for LB resistance. In the screening experiment, the parental lines, $F_1$ progeny, and the control genotypes NC 84173, New Yorker, NC 63EB, NC 870, and NC 03220 were included. The plants were grown in 72-cell seedling trays in an environmentally controlled greenhouse compartment. Parental, $F_1$, and control genotypes were grown in four replications and each replicate consisted of six individuals. Replicates were placed on opposite ends and sides of the greenhouse. When the plants were approximately six weeks old, the RH was increased to 95-100% using high-pressure foggers and the temperature was reduced to

16-18 ºC. Blackout curtains were lowered to reduce ambient light to suppress the hypersensitive and salicylic acid defense responses (GRIEBEL AND ZEIER 2008; RODEN AND INGLE 2009). Clear plastic was draped around each bench to prevent direct water accumulation on the plants. After approximately six hours, the high-pressure foggers were turned off temporarily and the plants were gently misted with water. After 30 minutes, the plants were uniformly sprayed with *P. infestans* inoculum using a concentration of 10,000 sporangia/mL and volume of 1 L/1000 plants. Thirty minutes later, a second application of inoculum was applied and the high-pressure foggers were reinitiated. After approximately 24 hours, the blackout curtains were removed to allow ambient lighting with no supplemental light.

The $F_2$ population was evaluated seven days after inoculation for foliar disease severity (% DS) on a scale of 0-100%. A score of 0% indicated no signs of LB infection and 100% indicated no remaining healthy tissue or complete defoliation. Each $F_2$ individual was assigned a single % DS score, while each parental, $F_1$, and control replicate was visually assigned a mean value based on the overall health of all six plants. The most resistant (n = 63, % DS <20) and susceptible (n = 36, % DS >85) plants were identified and retained, grown to maturity, and self-pollinated to produce $F_3$ progeny seed. Tissue was collected from all $F_2$ individuals and stored at -80 ºC for DNA extraction.

Resistance or susceptibility of the selected $F_2$ individuals was confirmed by evaluating the % DS of corresponding $F_3$ progeny families in four separate experiments (I, II, III, and IV). Screening methods for the $F_2$ and $F_3$ experiments were nearly identical apart from the following variations. Experiments I, II and III were conducted in two

similar and adjacent greenhouse sections, each containing one of two $F_3$ progeny family replicates. Experiment IV was conducted in a single greenhouse compartment and replicates for each family were placed on opposite sides of the greenhouse compartment. Experiment I was evaluated six days after inoculation and experiment II was evaluated after seven days. Experiments III and IV were each evaluated after five days. $F_3$ individuals within each family were each assigned a % DS score and the mean was calculated for each $F_3$ progeny family. Resistant $F_2$ parents of $F_3$ progeny that averaged <30% DS (n = 39) and susceptible $F_2$ parents of $F_3$ progeny families that averaged >70% DS (n = 35) were selected for marker genotyping. Due to insufficient $F_3$ seed, not all families were included in all experiments. However, $F_3$ progeny families of $F_2$ individuals selected for genotyping were evaluated in at least two experiments.

**Marker development**

DNA was extracted from parental genotypes, PI 163245 and Fla. 8059 using the DNeasy Plant Mini Kit (Qiagen, Seoul, Korea) following the manufacturers protocol. Reduced representation libraries (RRLs) were constructed based on a previously developed genotyping-by-sequencing (GBS) protocol (ELSHIRE *et al.* 2011). Briefly, DNA from the two parents was plated with adapter pairs and digested with the restriction enzyme, *NlaIII*. Ligation of bar-coded adapters was performed and the DNA was pooled and purified. Restriction fragments (RFs) were amplified via PCR and purified for a second time. Fragment sizes were determined and sequenced if the majority of DNA fragments fell between 170-350 bp. The genomic libraries were prepared and sequenced via paired-end sequencing using an Illumina Hi-seq 2000 by BGI@UC Davis.

The FASTQ sequencing files were de-multiplexed via custom Perl scripts and the reads were mapped to the tomato genome SL2.50 using CLC Genomics Workbench (WWW.CLCBIO.COM). An issue with one of the adapter sequences necessitated single-end alignment for mapping reads to the tomato reference genome. SNPs were called between PI 163245 and Fla. 8059 using SAMtools (LI *et al.* 2009). Only SNPs with at least three reads per genotype and no other mutations within 50 bp of the polymorphic locus were considered for use in this study. A subset of SNPs (n = 373) was selected for marker development and SNP validation.

**$F_2$ DNA extraction**

Genomic DNA extractions were performed using a modified quick extraction protocol (KING *et al.* 2014). For each $F_2$ sample, tomato leaf tissue 2-3 cm in diameter was collected and placed in 160 µl NaCl (5 M) and 240 µl extraction buffer (200 mM Tris/HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS). The tissue was pulverized using the Qiagen TissueLyser II or manually ground using a sterile plastic micropestle until no visible clumps remained. The samples were then incubated at 60 ºC for 30 minutes. After incubation, the samples were centrifuged for 5 minutes at 2,500 × *g* and the supernatant was combined with an equal volume of -20 ºC isopropanol. The solution was inverted several times to mix and then incubated for 15 minutes at -20 ºC before being centrifuged for 5 minutes at 2,500 × *g*. The samples were carefully decanted and 200 µl 70% ethanol was added to each DNA sample. The samples were centrifuged a final time at 2,500 × *g* for 5 minutes and the ethanol was decanted. The samples were either inverted and air dried at room temperatures or placed at 60 ºC until all ethanol had evaporated. The DNA pellets were re-suspended in Tris/EDTA (10 mM Tris, 1 mM

EDTA). DNA concentrations were quantified using a NanoDrop 2000 (THERMOSCIENTIFIC) and adjusted to between 30 and 60 ng/mL for Kompetitive Allele Specific PCR (KASP) genotyping.

**Genotyping**

The 373 SNPs were tested on each parent as well as $F_1$ progeny. Of the 373 SNPs selected for marker development, 233 SNPs, which were distributed throughout the tomato genome, were selected based on genomic location, validation of polymorphism, and consistency of fluorescent clustering. These markers were used to genotype the selected resistant and susceptible $F_2$ individuals for developing a genetic map and conducting trait-based QTL mapping. All KASP assay development and genotyping was performed at Ag-biotech, Monterey, CA, USA.

**Genetic map development**

Although all SNPs were physically mapped, a genetic linkage map was developed to confirm their relative locations and the genetic distances between markers. The linkage map was constructed using MapMaker 3.0 (LANDER *et al.* 1987). Using the GROUP command, the SNP markers were assigned to linkage groups using a LOD threshold of 3.0. The ORDER command was used to determine the correct marker orientation and verified using the RIPPLE command.

**Trait-based analysis (TBA) and other statistics**

Identification of genomic regions associated with LB resistance was done using TBA. For each marker locus (SNP), the allele frequencies of selected resistant and susceptible $F_2$ individuals were calculated (for a total of 233 SNPs). The marker allele frequency differences between the selected resistant and susceptible classes were

determined and compared with the standard error ($\sigma_p$) of the allele frequency difference at each corresponding locus (SNP). The following binomial equation was used to determine $\sigma_p$:

$$\sigma_p = (p_R q_R / 2N_R + p_S q_S / 2N_S)^{0.5},$$

where $p_R$ is the marker allele frequency of PI 163245 in the resistant class, $p_S$ is the marker allele frequency of PI 163245 in the susceptible class, $q_R$ is the Fla. 8059 marker allele frequency in the resistant class, $q_S$ is the marker allele frequency in the susceptible class, and $N_R$ and $N_S$ are the numbers of individuals in the resistant and susceptible classes, respectively. An allele frequency difference $\geq 2\sigma_p$ between the two selected classes was considered statistically significant, providing at least 95% confidence that a particular marker was associated with resistance (STEEL AND TORRIE 1980; LEBOWITZ *et al.* 1987).

For each marker, a chi-square ($\chi^2$) goodness-of-fit test was performed within each phenotypic class to determine whether marker segregation fit the expected 1:2:1 Mendelian ratio.

Mean comparisons of the parental, $F_1$, and resistant and susceptible control lines were calculated using Tukey's HSD test.

## Results

### Disease response of parental and control genotypes

The late blight disease severity (% DS) was evaluated for parental, $F_1$, and resistant and susceptible control lines in a total of five experiments. The LB resistance in the parental accession PI 163245 was moderately high, averaging 15.5% DS, and was not statistically different than cultivars containing resistance genes *Ph-2* (17.5% DS) and *Ph-*

*3* (9.8% DS). NC 03220, containing both *Ph-2* and *Ph-3*, was consistently the most resistant control line and averaged 4.3% DS. In contrast, the LB susceptible tomato breeding line, Fla. 8059, was highly susceptible (91.9% DS) and statistically similar to the LB susceptible control lines NC 84173 (92.8% DS) and New Yorker (93.3% DS). The $F_1$ generation displayed moderate susceptibility to LB infection (49.6% DS), similar to the mid-parent value (53.7% DS) (Table 4-1).

**Table 4-1** Response to late blight infection as a measure of percent disease severity (% DS) for parental and control lines, and $F_1$, $F_2$, and $F_3$ generations.

| Genotype | Number of plants or families | % DS[1] | Range (% DS) |
|---|---|---|---|
| $P_1$ (Fla. 8059) | 192 | $91.9 \pm 9.1^{a}$ | 50-98 |
| $P_2$ (PI 163245) | 186 | $15.5 \pm 9.4^{cd}$ | 5-40 |
| $F_1$ | 72 | $49.6 \pm 14.6^{b}$ | 25-80 |
| NC 84173 | 72 | $92.8 \pm 3.4^{a}$ | 90-98 |
| New Yorker (*Ph-1*) | 192 | $93.3 \pm 6.1^{a}$ | 70-100 |
| NC 63EB (*Ph-2*) | 192 | $17.5 \pm 9.6^{c}$ | 5-60 |
| NC 870 (*Ph-3*) | 162 | $9.8 \pm 4.5^{de}$ | 5-20 |
| NC 03220 (*Ph-2* + *Ph-3*) | 168 | $4.3 \pm 2.5^{e}$ | 0-10 |
| $F_2$ population | 560 | 51.9 ± 30.2 | 0-100 |
| $F_2$ susceptible class | 35 | 90.4 ± 3.2 | 85-100 |
| $F_2$ resistant class | 39 | 7.5 ± 5.0 | 0-20 |
| $F_3$ susceptible class families | 35 | 86.0 ± 7.9 | 5-100 |
| $F_3$ resistant class families | 39 | 20.4 ± 5.0 | 0-100 |

[1]Mean comparison of parental and control lines were determined using Tukey's HSD test and are denoted as by superscript (a-e).

### $F_2$ and $F_3$ response to LB infection

The % DS in the $F_2$ population (n = 560) averaged 51.9%, ranging from 0-100% DS (Table 4-1, Fig. 4-1). The distribution of $F_2$ progeny was non-normal, as determined by the Shapiro-Wilk test, and skewed slightly towards higher % DS (skewness = -0.194). The phenotypic distribution was somewhat bimodal including 319 individuals with ≥50% DS and 241 individuals with <50% DS (Fig. 4-1).

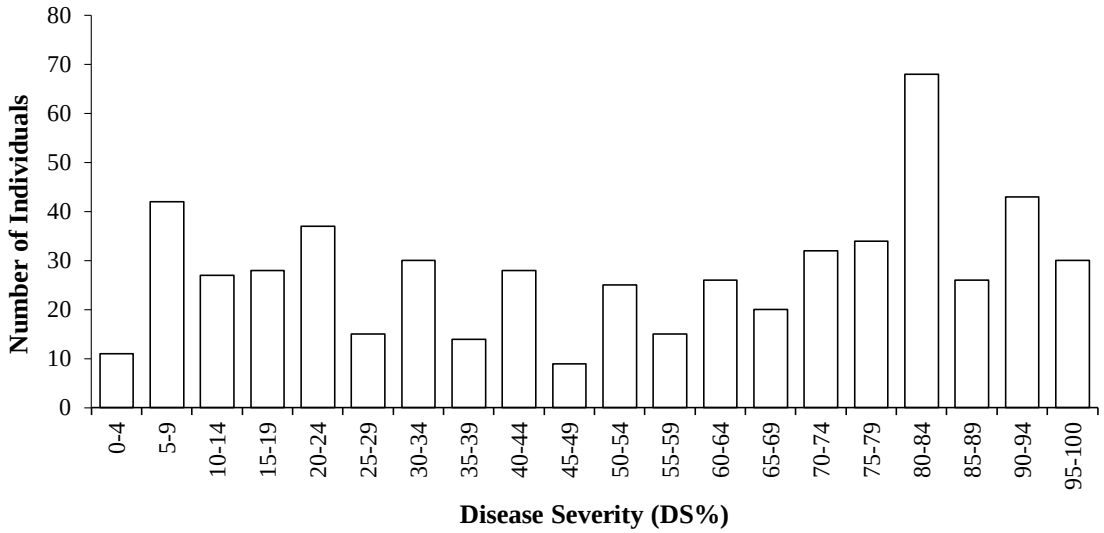



**Figure 4-1** Frequency distribution of disease severity (% DS) for an $F_2$ mapping population (n = 560) derived from a cross between Fla. 8059 and PI 163245. Foliar % DS was measured as a percentage of whole plant foliar disease symptoms/defoliation on a scale of 0-100% DS.

The % DS in the selected resistant $F_2$ individuals ranged from 0-20%, averaging 7.5%, while their $F_3$ progeny family means averaged 20.4% DS and ranged from 11.5-29.3% DS. In contrast, the selected susceptible $F_2$ individuals fell between 85-97% DS, averaging 90.4% and their $F_3$ progeny families averaged between 70.7-97.9% DS with an overall average of 86.0% (Table 4-1, Fig. 4-2).

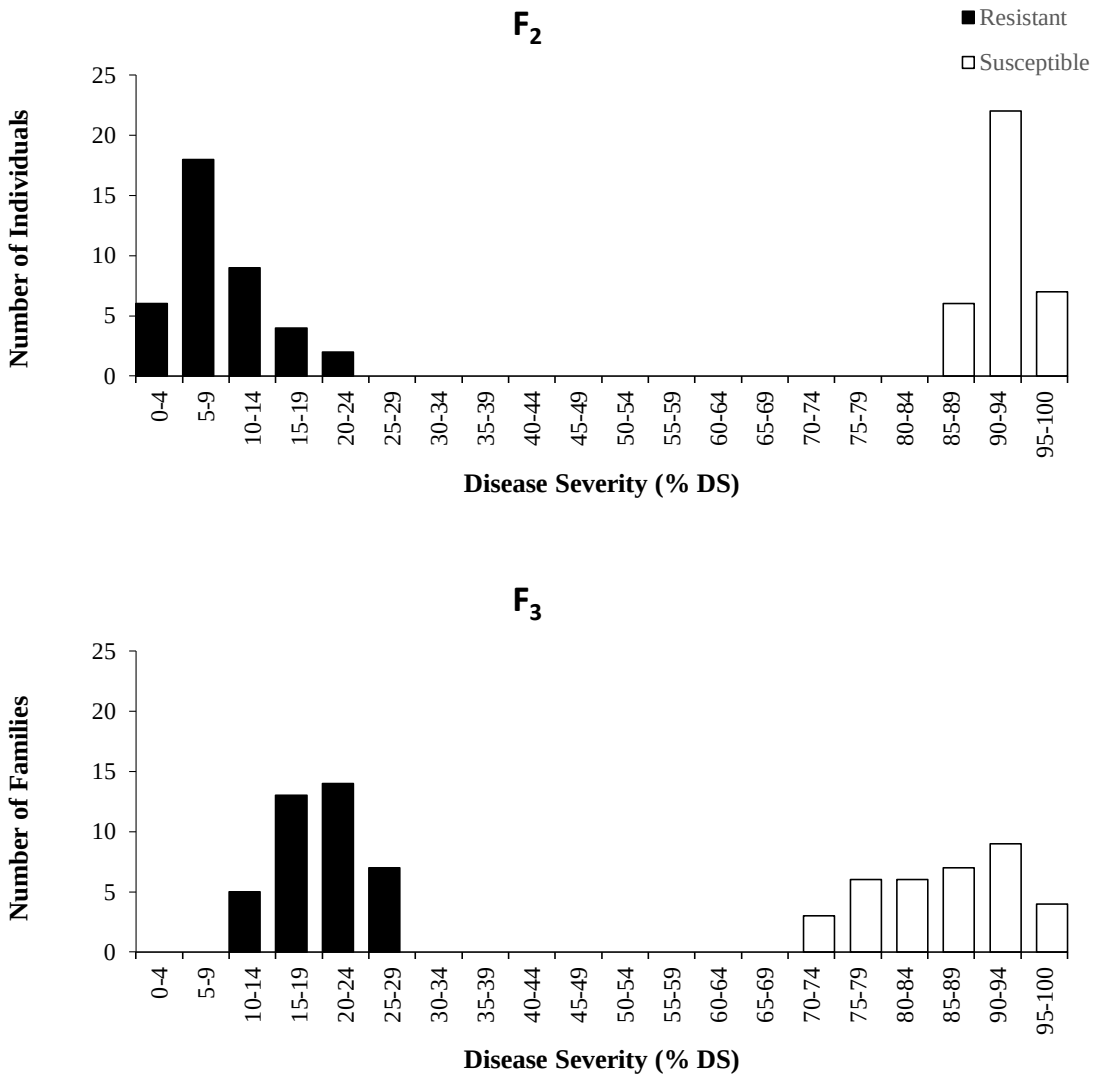



**Figure 4-2** Distributions of percent disease severity (% DS) for genotyped $F_2$ individuals (top) and the mean % DS of their $F_3$ progeny families (bottom). Foliar disease severity was measured on a scale of 0-100%.

## Marker discovery and validation

The Fla. 8059 RRL sequence overall covered approximately 27% of the reference genome. The sequencing depth across the entire genome was 1.29. However, by excluding zero-coverage regions, the average sequencing depth increased to 4.68. Zero-coverage distances averaged 462 bp and the maximum distance was slightly over 3 Mbp.

In total, sequencing of Fla. 8059 generated 11,285,819 reads and 74.9% were successfully mapped to the reference genome.

The PI 163245 sequencing results were similar to those of Fla. 8059, though in general the coverage and sequencing depth were slightly lower. Sequencing of PI 163245 covered 25% of the reference genome with an average read depth of 1.07. Excluding zero-coverage regions, the average read depth increased to 4.21. The mean length of zero-coverage regions was 473.9 bp and the maximum distance was just over 3 Mbp. A total of 9,310,064 reads were generated. Only 52.4% of reads were successfully mapped to the reference genome, perhaps stemming from higher dissimilarity between *S. pimpinellifolium* and the reference cultivated tomato genome.

Comparison of the Fla. 8059 and PI 163245 RRL sequences identified 33,385 SNPs distributed across the 12 tomato chromosomes. The largest numbers of SNPs were located on chromosome 11, accounting for nearly a third of all SNPs, and the fewest markers were found on chromosome 8. The majority of chromosomes averaged <50 kb between SNP markers, however chromosomes 4, 7, and 8 each averaged >100 kb between SNPs (Table 4-2).

**Table 4-2** Polymorphic SNPs between late blight (LB) susceptible Fla. 8059 and LB resistant PI 163245. SNPs were called using SAMtools (Li *et al.* 2009) and at least three reads per genotype were required with no additional mutations within 50 bp on either side of the given locus.

| Chromosome | Total SNPs | Average physical distance (bp) | Total physical distance (bp) |
|---|---|---|---|
| 1 | 2,464 | 39,949 | 98,433,159 |
| 2 | 1,784 | 30,876 | 55,082,228 |
| 3 | 1,516 | 46,594 | 70,636,951 |
| 4 | 633 | 104,413 | 66,093,287 |
| 5 | 3,071 | 21,425 | 65,797,473 |
| 6 | 1,184 | 41,772 | 49,457,639 |
| 7 | 655 | 103,553 | 67,827,469 |
| 8 | 566 | 116,306 | 65,829,433 |
| 9 | 3,022 | 23,581 | 71,261,811 |
| 10 | 6,691 | 9,783 | 65,458,676 |
| 11 | 9,319 | 5,986 | 55,781,368 |
| 12 | 2,480 | 26,997 | 66,951,748 |
| **Total** | 33,385 | - | 798,611,242 |

In total, 373 SNPs were selected for marker development and KASP markers were successfully developed for 261 (70.0%). Of the 261 markers, 233 were selected for genetic mapping of the selected resistant and susceptible $F_2$ individuals based on their physical and genetic map positions to ensure uniform genome coverage. Only four of the 261 SNPs which were successfully converted to KASP markers were not polymorphic between Fla. 8059 and PI 163245, validating 98.5% of SNPs that were selected for marker development for these two parental lines.

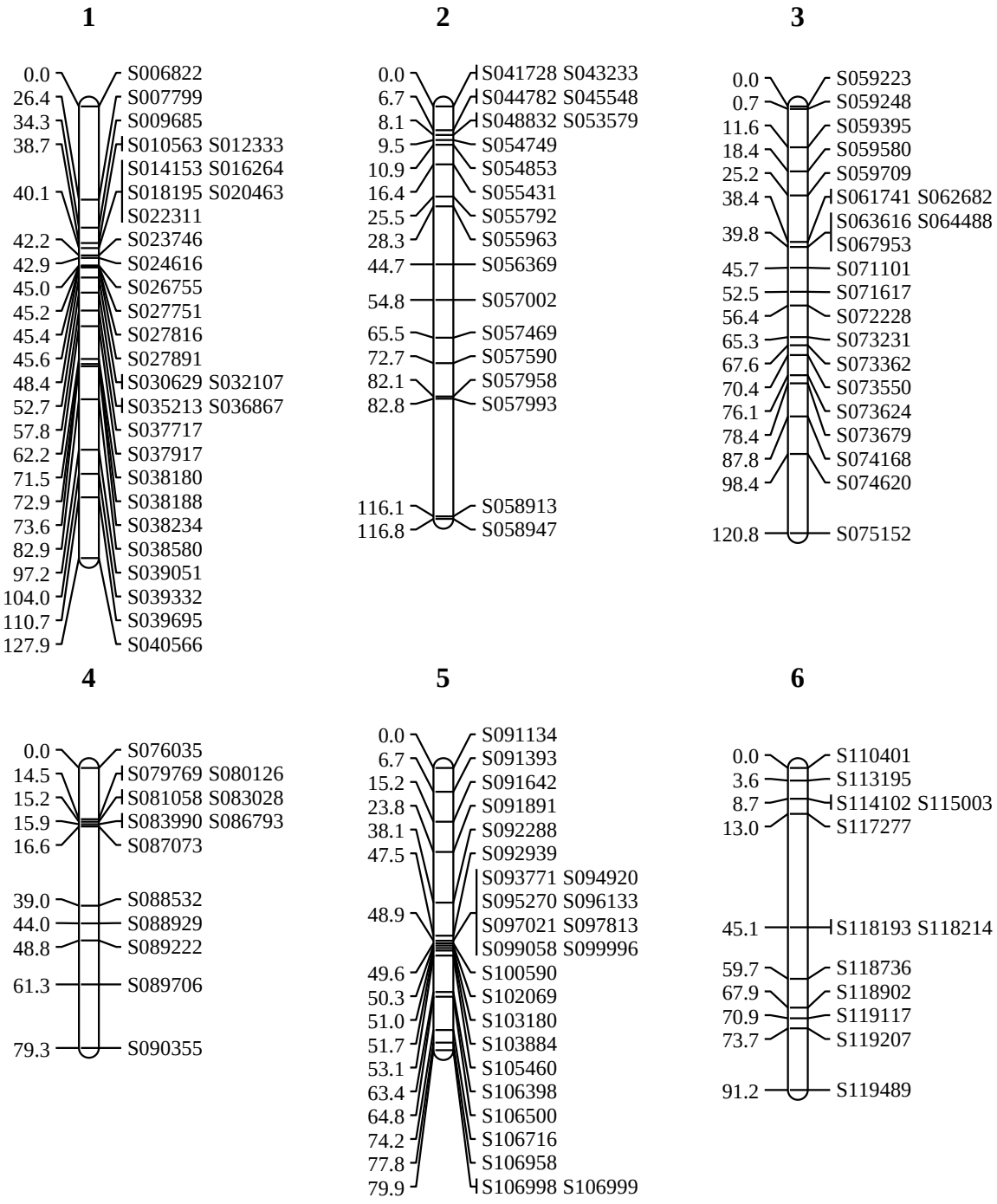
**Genetic map construction**

Genetic mapping identified 12 linkage groups corresponding to the 12 tomato chromosomes. The linkage groups ranged from 79.3-144.2 cM in length and the overall genome size was 1,278.2 cM. The number of SNPs on each chromosome ranged from 12-30 SNPs and the average distance between markers was 3.4 Mbp. The genetic distance between markers averaged 5.5 cM and for each linkage group (chromosome) the genetic distance between markers averaged from 3.2-7.8 cM (Table 4-3).

**Table 4-3** Genetic mapping of SNP markers (n = 233) in an $F_2$ mapping population (n = 74). Individuals were genotyped using KASP assays and mapped using MapMaker 3.0 (LANDER *et al.* 1987). The average genetic and physical distances between markers are provided.

| Chromosome | Chromosome Length (cM) | SNPs Genotyped | Average physical distance (bp) | Average Genetic distance (cM) |
|---|---|---|---|---|
| 1 | 127.9 | 30 | 3,100,716 | 4.3 |
| 2 | 116.8 | 19 | 2,806,690 | 6.1 |
| 3 | 120.8 | 21 | 3,313,405 | 5.8 |
| 4 | 79.3 | 13 | 4,651,120 | 6.1 |
| 5 | 79.9 | 25 | 2,548,258 | 3.2 |
| 6 | 91.2 | 12 | 3,165,957 | 7.6 |
| 7 | 108.8 | 15 | 4,377,659 | 7.3 |
| 8 | 93.2 | 12 | 5,278,031 | 7.8 |
| 9 | 102.3 | 20 | 3,422,624 | 5.1 |
| 10 | 117.1 | 21 | 3,065,052 | 5.6 |
| 11 | 96.7 | 22 | 2,361,656 | 4.4 |
| 12 | 144.2 | 23 | 2,823,015 | 6.3 |
| **Total** | 1,278.2 | 233 | 3,410,101 | 5.5 |

Genetic mapping indicated generally uniform coverage throughout the genome. The largest genetic distance between markers was 33.3 cM, located on chromosome 3. Only three additional genetic gaps >30 cM were identified, found on chromosomes 6, 7, and 12. Additionally, 12 genetic gaps between 20 cM and 30 cM were found. However, overall more than 80% of the genetic distances between markers were <10 cM (Fig. 4-3).


1
0.0 S006822
26.4 S007799
34.3 S009685
38.7 S010563 S012333
S014153 S016264
40.1 S018195 S020463
S022311
42.2 S023746
42.9 S024616
45.0 S026755
45.2 S027751
45.4 S027816
45.6 S027891
48.4 S030629 S032107
52.7 S035213 S036867
57.8 S037717
62.2 S037917
71.5 S038180
72.9 S038188
73.6 S038234
82.9 S038580
97.2 S039051
104.0 S039332
110.7 S039695
127.9 S040566
2
0.0 S041728 S043233
6.7 S044782 S045548
8.1 S048832 S053579
9.5 S054749
10.9 S054853
16.4 S055431
25.5 S055792
28.3 S055963
44.7 S056369
54.8 S057002
65.5 S057469
72.7 S057590
82.1 S057958
82.8 S057993
116.1 S058913
116.8 S058947
3
0.0 S059223
0.7 S059248
11.6 S059395
18.4 S059580
25.2 S059709
38.4 S061741 S062682
S063616 S064488
39.8 S067953
45.7 S071101
52.5 S071617
56.4 S072228
65.3 S073231
67.6 S073362
70.4 S073550
76.1 S073624
78.4 S073679
87.8 S074168
98.4 S074620
120.8 S075152
4
0.0 S076035
14.5 S079769 S080126
15.2 S081058 S083028
15.9 S083990 S086793
16.6 S087073
39.0 S088532
44.0 S088929
48.8 S089222
61.3 S089706
79.3 S090355
5
0.0 S091134
6.7 S091393
15.2 S091642
23.8 S091891
38.1 S092288
47.5 S092939
S093771 S094920
S095270 S096133
48.9 S097021 S097813
S099058 S099996
49.6 S100590
50.3 S102069
51.0 S103180
51.7 S103884
53.1 S105460
63.4 S106398
64.8 S106500
74.2 S106716
77.8 S106958
79.9 S106998 S106999
6
0.0 S110401
3.6 S113195
8.7 S114102 S115003
13.0 S117277
45.1 S118193 S118214
59.7 S118736
67.9 S118902
70.9 S119117
73.7 S119207
91.2 S119489

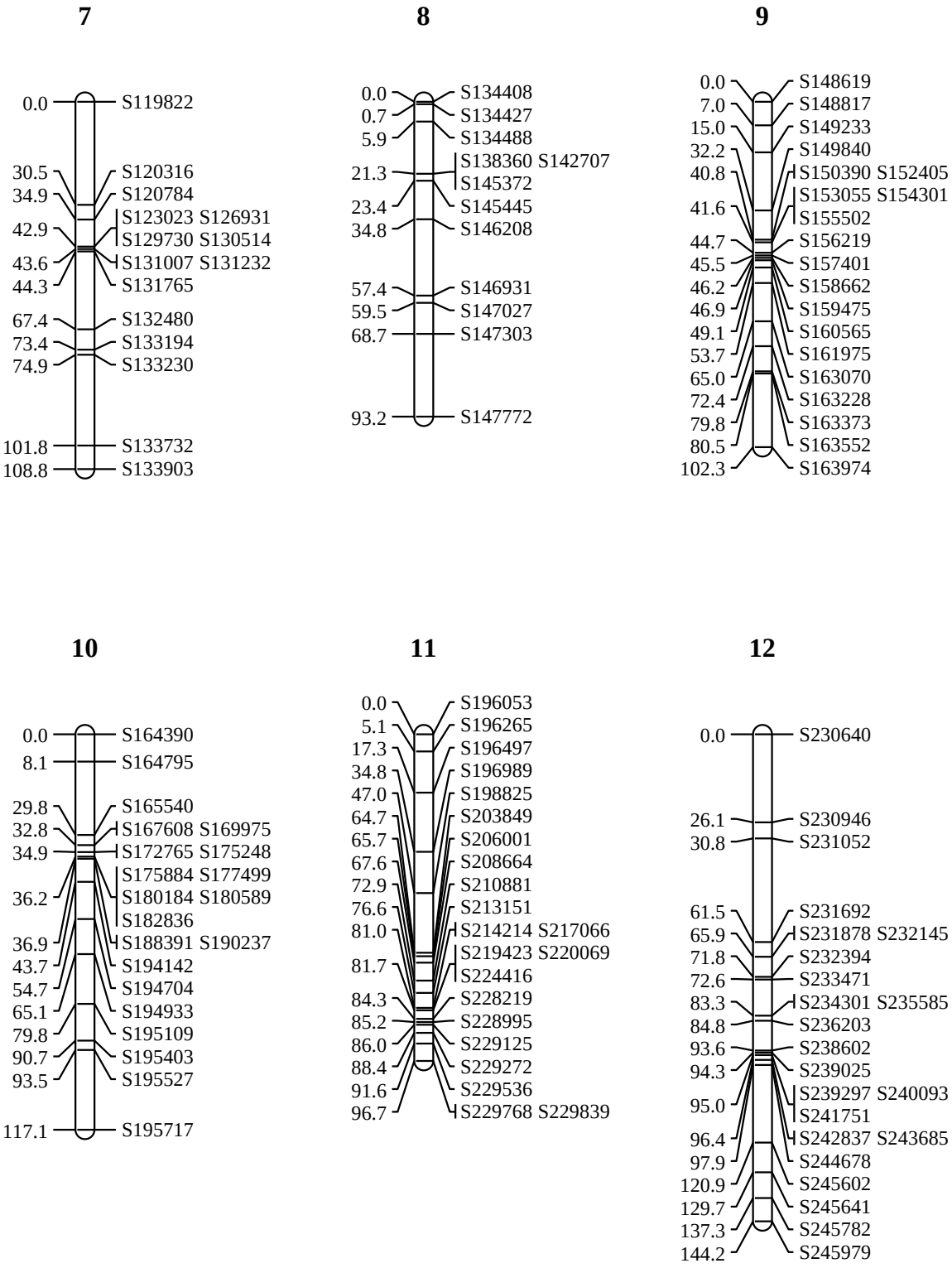



**Figure 4-3** $F_2$ genetic linkage map derived from a cross between Fla. 8059 and PI 163245. A total of 74 $F_2$ individuals were genotyped with 233 SNP markers. Genetic mapping was performed using MapMaker 3.0 (LANDER *et al.* 1987) and represented visually using MapChart 2.2 (VOORRIPS 2002).

**Marker segregation**

Chi-square analyses within the resistant and susceptible classes indicated several markers did not segregate in the expected 1:2:1 ratio. In the resistant class, 34 markers significantly deviated from the expected, while in the susceptible class 31 markers exhibited skewed segregation. In the resistant class, seven SNPs on the long arm of chromosome 1, five on chromosome 10, and one on each of chromosomes 11 and 12 were significantly skewed in favor of PI 163245 alleles. The last marker on chromosome 4 was skewed slightly in favor of the Fla. 8059 and the heterozygous genotype was overrepresented. Additionally, heterozygous genotypes for four markers on chromosome 5 and one marker on chromosome 10 were more frequent than expected. There were several instances within the resistant class in which segregation was skewed in favor of Fla. 8059. The Fla. 8059 genotype was overrepresented for four markers on chromosome 7 and five markers on chromosomes 9 and 11 (Table 4-4).

In the susceptible class, nine markers at the top of chromosome 2 were significantly skewed in favor of the susceptible parental genotype. Additionally, five markers on chromosome 10 and two markers on chromosome 11 were both skewed in favor of Fla. 8059. Similar to the resistant class, at several markers the heterozygous genotypes were more frequent than expected. In the susceptible class, segregation favored the heterozygous genotype for one marker on chromosome 7 and seven markers on chromosome 9. There were a few instances where segregation in the susceptible class was skewed in favor of the PI 163245 allele. Three markers on chromosome 4 were significantly skewed in favor of the *S. pimpinellifolium* allele. This was also observed for the first two markers on chromosome 9 and two markers on chromosome 12 (Table 4-4).

Only nine markers with skewed segregation occurred in both the resistant and susceptible classes. These markers were located on chromosomes 9, 10, 11, and 12. The first two markers on chromosome 9 were skewed in opposite directions, with the Fla. 8059 genotype more highly represented in the resistant class. Genotypes for the last five markers on chromosome 10 also segregated in opposite directions between classes, in this case heavily favoring PI 163245 in the resistant class. One region on chromosome 11 favored the Fla. 8059 genotype in both the resistant and susceptible classes, while a single marker on chromosome 12 favored PI 163245 in both classes (Table 4-4).

**Table 4-4** Marker locations and segregation in an $F_2$ mapping population (n = 560) derived from a cross of Fla. 8059 and PI 163245. Highly resistant (n = 39) and susceptible (n = 35) $F_2$ individuals were genotyped with 233 markers. For each SNP marker, genotypes correspond to *pp* (homozygous PI 163245), *pq* (heterozygous), and *qq* (homozygous Fla. 8059). Chi-square ($X^2$) analyses were performed at each locus to determine if the genotype frequencies fit the expected 1:2:1 Mendelian ratio for an $F_2$ population.

| Marker | Chromosome | Physical Locus (bp) | Genetic Locus (cM) | Resistant Class | | | | Susceptible class | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *pp* | *pq* | *qq* | $X^2$ (1:2:1) | *pp* | *pq* | *qq* | $X^2$ (1:2:1) |
| S006822 | 1 | 869,553 | 0.0 | 11 | 18 | 10 | 0.28 | 11 | 11 | 13 | 5.06 |
| S007799 | 1 | 4,335,049 | 26.4 | 7 | 23 | 5 | 3.69 | 7 | 19 | 7 | 0.76 |
| S009685 | 1 | 9,985,745 | 34.3 | 4 | 18 | 11 | 3.24 | 7 | 20 | 8 | 0.77 |
| S010563 | 1 | 12,048,262 | 38.7 | 8 | 23 | 7 | 1.74 | 7 | 21 | 6 | 1.94 |
| S012333 | 1 | 15,590,570 | 38.7 | 8 | 22 | 8 | 0.95 | 7 | 21 | 7 | 1.40 |
| S014153 | 1 | 19,213,673 | 40.1 | 8 | 24 | 7 | 2.13 | 7 | 22 | 6 | 2.37 |
| S016264 | 1 | 23,130,726 | 40.1 | 8 | 24 | 7 | 2.13 | 7 | 22 | 6 | 2.37 |
| S018195 | 1 | 27,074,245 | 40.1 | 8 | 24 | 7 | 2.13 | 7 | 22 | 6 | 2.37 |
| S020463 | 1 | 31,362,495 | 40.1 | 8 | 22 | 7 | 1.38 | 7 | 22 | 6 | 2.37 |
| S022311 | 1 | 39,849,852 | 40.1 | 8 | 24 | 7 | 2.13 | 7 | 22 | 6 | 2.37 |
| S023746 | 1 | 42,993,171 | 42.2 | 8 | 20 | 9 | 0.30 | 7 | 21 | 6 | 1.94 |
| S024616 | 1 | 45,193,611 | 42.9 | 3 | 17 | 8 | 3.07 | 4 | 15 | 5 | 1.58 |
| S026755 | 1 | 48,378,876 | 45.0 | 8 | 24 | 7 | 2.13 | 7 | 21 | 6 | 1.94 |
| S027751 | 1 | 51,542,987 | 45.2 | 8 | 23 | 7 | 1.74 | 7 | 20 | 7 | 1.06 |
| S027816 | 1 | 51,992,555 | 45.4 | 8 | 22 | 6 | 2.00 | 7 | 20 | 6 | 1.55 |
| S027891 | 1 | 52,490,187 | 45.6 | 8 | 22 | 8 | 0.95 | 6 | 20 | 7 | 1.55 |
| S030629 | 1 | 56,569,777 | 48.4 | 5 | 25 | 7 | 4.78 | 6 | 22 | 6 | 2.94 |
| S032107 | 1 | 59,530,472 | 48.4 | 5 | 23 | 8 | 3.28 | 6 | 22 | 5 | 3.73 |
| S035213 | 1 | 67,053,483 | 52.7 | 8 | 22 | 9 | 0.69 | 7 | 22 | 6 | 2.37 |
| S036867 | 1 | 72,679,941 | 52.7 | 8 | 20 | 9 | 0.30 | 7 | 21 | 5 | 2.70 |
| S037717 | 1 | 76,215,183 | 57.8 | 11 | 21 | 7 | 1.05 | 7 | 19 | 8 | 0.53 |
| S037917 | 1 | 77,444,743 | 62.2 | 12 | 23 | 3 | 5.95 | 8 | 18 | 8 | 0.12 |
| S038180 | 1 | 79,762,738 | 71.5 | 15 | 21 | 3 | 7.62* | 11 | 16 | 8 | 0.77 |
| S038188 | 1 | 79,855,761 | 72.9 | 16 | 15 | 3 | 10.41* | 12 | 11 | 8 | 3.65 |
| S038234 | 1 | 80,145,027 | 73.6 | 15 | 19 | 3 | 7.81* | 11 | 16 | 8 | 0.77 |
| S038580 | 1 | 82,841,484 | 82.9 | 14 | 22 | 2 | 8.53* | 12 | 16 | 6 | 2.24 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S039051 | 1 | 86,022,035 | 97.2 | 13 | 22 | 1 | 9.78* | 11 | 17 | 7 | 0.94 |
| S039332 | 1 | 87,665,965 | 104.0 | 12 | 25 | 2 | 8.23* | 10 | 17 | 7 | 0.53 |
| S039695 | 1 | 89,731,813 | 110.7 | 14 | 23 | 2 | 8.64* | 12 | 16 | 7 | 1.69 |
| S040566 | 1 | 93,891,040 | 127.9 | 12 | 22 | 5 | 3.15 | 11 | 13 | 11 | 2.31 |
| S041728 | 2 | 537,665 | 0.0 | 10 | 14 | 12 | 2.00 | 2 | 10 | 16 | 16.29* |
| S043233 | 2 | 3,416,012 | 0.0 | 10 | 17 | 12 | 0.85 | 2 | 15 | 14 | 9.32* |
| S044782 | 2 | 6,233,893 | 6.7 | 12 | 18 | 7 | 1.38 | 3 | 16 | 14 | 7.36* |
| S045548 | 2 | 7,421,251 | 6.7 | 11 | 17 | 7 | 0.94 | 3 | 17 | 14 | 7.12* |
| S048832 | 2 | 15,226,512 | 8.1 | 12 | 20 | 7 | 1.31 | 2 | 16 | 15 | 10.27* |
| S053579 | 2 | 25,809,518 | 8.1 | 11 | 16 | 6 | 1.55 | 3 | 16 | 14 | 7.36* |
| S054749 | 2 | 31,192,221 | 9.5 | 11 | 19 | 7 | 0.89 | 2 | 15 | 15 | 10.69* |
| S054853 | 2 | 32,920,145 | 10.9 | 12 | 19 | 7 | 1.32 | 5 | 12 | 17 | 11.41* |
| S055431 | 2 | 34,968,684 | 16.4 | 10 | 12 | 9 | 1.65 | 4 | 14 | 14 | 6.75* |
| S055792 | 2 | 36,645,658 | 25.5 | 10 | 22 | 7 | 1.10 | 5 | 16 | 14 | 4.89 |
| S055963 | 2 | 37,415,171 | 28.3 | 9 | 21 | 8 | 0.47 | 6 | 16 | 13 | 3.06 |
| S056369 | 2 | 39,455,593 | 44.7 | 11 | 20 | 6 | 1.59 | 6 | 19 | 10 | 1.17 |
| S057002 | 2 | 42,403,413 | 54.8 | 8 | 21 | 6 | 1.63 | 6 | 18 | 9 | 0.82 |
| S057469 | 2 | 44,645,633 | 65.5 | 8 | 23 | 5 | 3.28 | 4 | 22 | 9 | 3.74 |
| S057590 | 2 | 45,456,951 | 72.7 | 8 | 24 | 6 | 2.84 | 4 | 23 | 8 | 4.37 |
| S057958 | 2 | 47,655,240 | 82.1 | 10 | 17 | 11 | 0.47 | 6 | 19 | 10 | 1.17 |
| S057993 | 2 | 47,840,923 | 82.8 | 9 | 17 | 12 | 0.89 | 6 | 19 | 10 | 1.17 |
| S058913 | 2 | 53,597,419 | 116.1 | 6 | 18 | 13 | 2.68 | 7 | 20 | 7 | 1.06 |
| S058947 | 2 | 53,864,779 | 116.8 | 6 | 19 | 13 | 2.58 | 7 | 20 | 8 | 0.77 |
| S059223 | 3 | 868,160 | 0.0 | 10 | 14 | 15 | 4.38 | 9 | 17 | 9 | 0.03 |
| S059248 | 3 | 1,012,927 | 0.7 | 10 | 12 | 15 | 5.92 | 8 | 16 | 9 | 0.09 |
| S059395 | 3 | 1,900,997 | 11.6 | 10 | 17 | 12 | 0.85 | 8 | 15 | 11 | 1.00 |
| S059580 | 3 | 2,842,514 | 18.4 | 8 | 15 | 14 | 3.27 | 8 | 15 | 9 | 0.19 |
| S059709 | 3 | 3,702,920 | 25.2 | 8 | 20 | 10 | 0.32 | 6 | 19 | 9 | 1.00 |
| S061741 | 3 | 19,906,458 | 38.4 | 9 | 20 | 8 | 0.30 | 7 | 21 | 7 | 1.40 |
| S062682 | 3 | 25,024,646 | 38.4 | 10 | 21 | 8 | 0.44 | 7 | 20 | 7 | 1.06 |
| S063616 | 3 | 28,874,679 | 39.8 | 9 | 20 | 9 | 0.11 | 7 | 21 | 7 | 1.40 |
| S064488 | 3 | 31,405,225 | 39.8 | 9 | 20 | 9 | 0.11 | 7 | 20 | 7 | 1.06 |
| S067953 | 3 | 40,915,234 | 39.8 | 9 | 21 | 9 | 0.23 | 7 | 20 | 7 | 1.06 |
| S071101 | 3 | 53,346,498 | 45.7 | 7 | 22 | 10 | 1.10 | 8 | 20 | 7 | 0.77 |
| S071617 | 3 | 55,700,401 | 52.5 | 6 | 22 | 9 | 1.81 | 7 | 14 | 11 | 1.50 |
| S072228 | 3 | 57,569,147 | 56.4 | 7 | 22 | 8 | 1.38 | 7 | 13 | 12 | 2.69 |
| S073231 | 3 | 60,755,534 | 65.3 | 9 | 21 | 8 | 0.47 | 4 | 18 | 11 | 3.24 |
| S073362 | 3 | 61,353,196 | 67.6 | 9 | 17 | 8 | 0.06 | 4 | 15 | 12 | 4.16 |
| S073550 | 3 | 62,408,550 | 70.4 | 9 | 22 | 7 | 1.16 | 5 | 17 | 12 | 2.88 |
| S073624 | 3 | 62,874,374 | 76.1 | 11 | 14 | 8 | 1.30 | 4 | 19 | 11 | 3.35 |
| S073679 | 3 | 63,035,518 | 78.4 | 11 | 21 | 6 | 1.74 | 4 | 19 | 12 | 3.91 |
| S074168 | 3 | 65,027,754 | 87.8 | 8 | 23 | 7 | 1.74 | 3 | 19 | 12 | 5.24 |
| S074620 | 3 | 67,012,384 | 98.4 | 9 | 21 | 8 | 0.47 | 4 | 17 | 12 | 3.91 |
| S075152 | 3 | 70,449,660 | 120.8 | 13 | 14 | 12 | 3.15 | 6 | 21 | 8 | 1.63 |
| S076035 | 4 | 4,474,495 | 0.0 | 8 | 13 | 13 | 3.35 | 8 | 21 | 5 | 2.41 |
| S079769 | 4 | 16,852,131 | 14.5 | 7 | 20 | 12 | 1.31 | 9 | 21 | 5 | 2.31 |
| S080126 | 4 | 18,403,976 | 14.5 | 7 | 19 | 12 | 1.32 | 9 | 21 | 5 | 2.31 |
| S081058 | 4 | 22,331,703 | 15.2 | 7 | 20 | 12 | 1.31 | 10 | 20 | 5 | 2.14 |
| S083028 | 4 | 31,773,887 | 15.2 | 7 | 17 | 10 | 0.53 | 10 | 19 | 5 | 1.94 |
| S083990 | 4 | 35,883,127 | 15.9 | 7 | 19 | 12 | 1.32 | 9 | 21 | 5 | 2.31 |
| S086793 | 4 | 47,086,022 | 15.9 | 7 | 20 | 12 | 1.31 | 9 | 21 | 5 | 2.31 |
| S087073 | 4 | 48,328,855 | 16.6 | 7 | 21 | 11 | 1.05 | 9 | 21 | 5 | 2.31 |
| S088532 | 4 | 55,822,031 | 39.0 | 7 | 18 | 11 | 0.89 | 11 | 23 | 1 | 9.17* |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S088929 | 4 | 59,296,245 | 44.0 | 5 | 21 | 11 | 2.62 | 11 | 20 | 3 | 4.82 |
| S089222 | 4 | 60,319,022 | 48.8 | 6 | 24 | 7 | 3.32 | 12 | 21 | 2 | 7.11* |
| S089706 | 4 | 62,522,878 | 61.3 | 5 | 22 | 10 | 2.68 | 12 | 20 | 2 | 6.94* |
| S090355 | 4 | 64,939,052 | 79.3 | 4 | 27 | 8 | 6.59* | 10 | 21 | 4 | 3.46 |
| S091134 | 5 | 452,980 | 0.0 | 10 | 19 | 10 | 0.03 | 7 | 18 | 10 | 0.54 |
| S091393 | 5 | 1,732,031 | 6.7 | 10 | 18 | 10 | 0.11 | 6 | 19 | 10 | 1.17 |
| S091642 | 5 | 3,179,941 | 15.2 | 8 | 18 | 10 | 0.22 | 7 | 18 | 8 | 0.33 |
| S091891 | 5 | 4,864,767 | 23.8 | 6 | 23 | 9 | 2.16 | 7 | 17 | 10 | 0.53 |
| S092288 | 5 | 6,847,590 | 38.1 | 6 | 27 | 6 | 5.77 | 7 | 18 | 10 | 0.54 |
| S092939 | 5 | 10,198,437 | 47.5 | 8 | 26 | 5 | 4.79 | 8 | 17 | 10 | 0.26 |
| S093771 | 5 | 14,119,126 | 48.9 | 8 | 25 | 5 | 4.26 | 9 | 15 | 11 | 0.94 |
| S094920 | 5 | 18,064,948 | 48.9 | 8 | 25 | 5 | 4.26 | 8 | 15 | 10 | 0.52 |
| S095270 | 5 | 20,441,367 | 48.9 | 8 | 26 | 5 | 4.79 | 9 | 15 | 11 | 0.94 |
| S096133 | 5 | 24,602,861 | 48.9 | 8 | 26 | 5 | 4.79 | 9 | 14 | 11 | 1.29 |
| S097021 | 5 | 29,610,944 | 48.9 | 6 | 26 | 5 | 6.14* | 9 | 15 | 11 | 0.94 |
| S097813 | 5 | 33,169,543 | 48.9 | 8 | 26 | 5 | 4.79 | 9 | 15 | 11 | 0.94 |
| S099058 | 5 | 36,717,287 | 48.9 | 8 | 25 | 5 | 4.26 | 8 | 15 | 11 | 1.00 |
| S099996 | 5 | 40,063,110 | 48.9 | 8 | 25 | 5 | 4.26 | 8 | 15 | 10 | 0.52 |
| S100590 | 5 | 43,797,160 | 49.6 | 7 | 27 | 5 | 5.97 | 9 | 15 | 11 | 0.94 |
| S102069 | 5 | 47,056,460 | 50.3 | 6 | 24 | 5 | 4.89 | 8 | 13 | 12 | 2.45 |
| S103180 | 5 | 50,124,659 | 51.0 | 8 | 24 | 5 | 3.76 | 8 | 14 | 12 | 2.00 |
| S103884 | 5 | 52,573,229 | 51.7 | 8 | 23 | 5 | 3.28 | 9 | 15 | 11 | 0.94 |
| S105460 | 5 | 59,122,546 | 53.1 | 7 | 26 | 5 | 5.37 | 10 | 15 | 10 | 0.71 |
| S106398 | 5 | 61,498,446 | 63.4 | 9 | 19 | 5 | 1.73 | 11 | 12 | 9 | 2.25 |
| S106500 | 5 | 61,668,015 | 64.8 | 8 | 25 | 6 | 3.31 | 11 | 15 | 9 | 0.94 |
| S106716 | 5 | 62,598,666 | 74.2 | 6 | 28 | 5 | 7.46* | 9 | 18 | 8 | 0.09 |
| S106958 | 5 | 63,969,295 | 77.8 | 6 | 28 | 5 | 7.46* | 9 | 18 | 7 | 0.35 |
| S106998 | 5 | 64,156,913 | 79.9 | 5 | 24 | 6 | 4.89 | 10 | 17 | 7 | 0.53 |
| S106999 | 5 | 64,159,419 | 79.9 | 5 | 28 | 6 | 7.46* | 10 | 18 | 7 | 0.54 |
| S110401 | 6 | 12,579,935 | 0.0 | 12 | 14 | 13 | 3.15 | 11 | 16 | 8 | 0.77 |
| S113195 | 6 | 20,181,291 | 3.6 | 9 | 14 | 12 | 1.91 | 7 | 19 | 4 | 2.73 |
| S114102 | 6 | 22,164,459 | 8.7 | 11 | 16 | 10 | 0.73 | 8 | 17 | 10 | 0.26 |
| S115003 | 6 | 25,472,372 | 8.7 | 12 | 17 | 10 | 0.85 | 8 | 17 | 10 | 0.26 |
| S117277 | 6 | 32,786,606 | 13.0 | 10 | 18 | 11 | 0.28 | 10 | 14 | 11 | 1.46 |
| S118193 | 6 | 38,750,383 | 45.1 | 7 | 17 | 13 | 2.19 | 8 | 23 | 4 | 4.37 |
| S118214 | 6 | 38,826,424 | 45.1 | 8 | 17 | 13 | 1.74 | 8 | 22 | 4 | 3.88 |
| S118736 | 6 | 41,601,855 | 59.7 | 9 | 18 | 10 | 0.08 | 9 | 19 | 6 | 1.00 |
| S118902 | 6 | 42,813,562 | 67.9 | 11 | 17 | 11 | 0.64 | 9 | 14 | 9 | 0.50 |
| S119117 | 6 | 44,204,004 | 70.9 | 11 | 17 | 11 | 0.64 | 10 | 16 | 9 | 0.31 |
| S119207 | 6 | 44,794,409 | 73.7 | 10 | 19 | 9 | 0.05 | 10 | 14 | 10 | 1.06 |
| S119489 | 6 | 47,405,467 | 91.2 | 10 | 25 | 4 | 4.95 | 9 | 22 | 4 | 3.74 |
| S119822 | 7 | 919,445 | 0.0 | 5 | 24 | 10 | 3.36 | 10 | 18 | 6 | 1.06 |
| S120316 | 7 | 4,133,718 | 30.5 | 9 | 16 | 12 | 1.16 | 11 | 16 | 7 | 1.06 |
| S120784 | 7 | 7,056,196 | 34.9 | 7 | 10 | 10 | 2.48 | 12 | 9 | 7 | 5.36 |
| S123023 | 7 | 17,266,353 | 42.9 | 3 | 25 | 10 | 6.37* | 8 | 20 | 7 | 0.77 |
| S126931 | 7 | 32,900,122 | 42.9 | 4 | 25 | 10 | 4.95 | 8 | 20 | 7 | 0.77 |
| S129730 | 7 | 44,901,162 | 42.9 | 4 | 20 | 10 | 3.18 | 8 | 17 | 7 | 0.19 |
| S130514 | 7 | 48,242,934 | 42.9 | 3 | 25 | 10 | 6.37* | 8 | 20 | 7 | 0.77 |
| S131007 | 7 | 50,199,194 | 43.6 | 4 | 23 | 10 | 4.14 | 9 | 19 | 7 | 0.49 |
| S131232 | 7 | 51,141,453 | 43.6 | 4 | 25 | 10 | 4.95 | 9 | 19 | 7 | 0.49 |
| S131765 | 7 | 54,254,472 | 44.3 | 4 | 22 | 11 | 3.97 | 9 | 19 | 7 | 0.49 |
| S132480 | 7 | 59,742,785 | 67.4 | 5 | 23 | 11 | 3.10 | 6 | 23 | 5 | 4.29 |
| S133194 | 7 | 60,971,609 | 73.4 | 5 | 21 | 13 | 3.51 | 6 | 22 | 7 | 2.37 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S133230 | 7 | 61,211,852 | 74.9 | 5 | 19 | 8 | 1.69 | 4 | 24 | 6 | 6.00* |
| S133732 | 7 | 65,251,571 | 101.8 | 1 | 16 | 19 | 18.44* | 6 | 17 | 12 | 2.09 |
| S133903 | 7 | 66,584,336 | 108.8 | 2 | 18 | 19 | 15.05* | 6 | 17 | 11 | 1.47 |
| S134408 | 8 | 1,675,976 | 0.0 | 11 | 19 | 9 | 0.23 | 5 | 23 | 6 | 4.29 |
| S134427 | 8 | 1,771,623 | 0.7 | 11 | 18 | 9 | 0.32 | 6 | 19 | 7 | 1.19 |
| S134488 | 8 | 2,284,340 | 5.9 | 8 | 21 | 8 | 0.68 | 6 | 22 | 7 | 2.37 |
| S138360 | 8 | 20,126,871 | 21.3 | 10 | 21 | 7 | 0.89 | 7 | 17 | 10 | 0.53 |
| S142707 | 8 | 38,970,761 | 21.3 | 10 | 21 | 6 | 1.54 | 7 | 17 | 10 | 0.53 |
| S145372 | 8 | 50,453,059 | 21.3 | 10 | 21 | 7 | 0.89 | 7 | 18 | 10 | 0.54 |
| S145445 | 8 | 50,817,661 | 23.4 | 11 | 17 | 7 | 0.94 | 9 | 16 | 10 | 0.31 |
| S146208 | 8 | 56,486,359 | 34.8 | 8 | 23 | 7 | 1.74 | 7 | 16 | 11 | 1.06 |
| S146931 | 8 | 60,561,857 | 57.4 | 9 | 22 | 6 | 1.81 | 7 | 15 | 13 | 2.77 |
| S147027 | 8 | 61,038,457 | 59.5 | 10 | 20 | 7 | 0.73 | 6 | 16 | 13 | 3.06 |
| S147303 | 8 | 62,457,271 | 68.7 | 8 | 18 | 7 | 0.33 | 4 | 20 | 11 | 3.51 |
| S147772 | 8 | 65,012,353 | 93.2 | 7 | 25 | 6 | 3.84 | 4 | 24 | 7 | 5.34 |
| S148619 | 9 | 1,489,519 | 0.0 | 4 | 19 | 16 | 7.41* | 16 | 12 | 7 | 8.09* |
| S148817 | 9 | 2,136,373 | 7.0 | 3 | 11 | 20 | 21.24* | 13 | 10 | 6 | 6.17* |
| S149233 | 9 | 3,645,130 | 15.0 | 5 | 13 | 17 | 10.54* | 13 | 14 | 6 | 3.73 |
| S149840 | 9 | 6,390,031 | 32.2 | 5 | 19 | 14 | 4.26 | 6 | 26 | 3 | 8.77* |
| S150390 | 9 | 9,102,976 | 40.8 | 6 | 22 | 8 | 2.00 | 5 | 25 | 4 | 7.59* |
| S152405 | 9 | 17,396,095 | 40.8 | 6 | 22 | 9 | 1.81 | 5 | 25 | 4 | 7.59* |
| S153055 | 9 | 22,152,953 | 41.6 | 6 | 23 | 8 | 2.41 | 5 | 24 | 4 | 6.88* |
| S154301 | 9 | 27,201,885 | 41.6 | 6 | 23 | 8 | 2.41 | 5 | 25 | 4 | 7.59* |
| S155502 | 9 | 31,264,469 | 41.6 | 6 | 23 | 8 | 2.41 | 5 | 25 | 4 | 7.59* |
| S156219 | 9 | 35,321,743 | 44.7 | 6 | 19 | 8 | 1.00 | 5 | 20 | 3 | 5.43 |
| S157401 | 9 | 39,364,039 | 45.5 | 6 | 19 | 12 | 1.97 | 4 | 23 | 6 | 5.36 |
| S158662 | 9 | 43,130,366 | 46.2 | 6 | 20 | 8 | 1.29 | 5 | 24 | 4 | 6.88* |
| S159475 | 9 | 45,478,987 | 46.9 | 6 | 19 | 12 | 1.97 | 5 | 23 | 5 | 5.12 |
| S160565 | 9 | 50,215,666 | 49.1 | 7 | 14 | 9 | 0.40 | 5 | 20 | 4 | 4.24 |
| S161975 | 9 | 57,608,575 | 53.7 | 6 | 23 | 9 | 2.16 | 5 | 24 | 5 | 5.76 |
| S163070 | 9 | 63,683,111 | 65.0 | 7 | 22 | 9 | 1.16 | 8 | 21 | 4 | 3.42 |
| S163228 | 9 | 64,829,240 | 72.4 | 8 | 9 | 13 | 6.47* | 6 | 14 | 8 | 0.29 |
| S163373 | 9 | 65,882,930 | 79.8 | 7 | 22 | 9 | 1.16 | 6 | 22 | 5 | 3.73 |
| S163552 | 9 | 67,064,767 | 80.5 | 7 | 21 | 10 | 0.89 | 7 | 21 | 7 | 1.40 |
| S163974 | 9 | 69,941,999 | 102.3 | 3 | 20 | 14 | 6.78* | 9 | 15 | 11 | 0.94 |
| S164390 | 10 | 41,931 | 0.0 | 5 | 23 | 9 | 3.05 | 12 | 20 | 3 | 5.34 |
| S164795 | 10 | 1,754,215 | 8.1 | 4 | 23 | 9 | 4.17 | 6 | 23 | 4 | 5.36 |
| S165540 | 10 | 4,829,839 | 29.8 | 7 | 18 | 11 | 0.89 | 7 | 21 | 5 | 2.70 |
| S167608 | 10 | 9,287,022 | 32.8 | 7 | 20 | 7 | 1.06 | 7 | 22 | 5 | 3.18 |
| S169975 | 10 | 13,045,160 | 32.8 | 3 | 20 | 10 | 4.45 | 7 | 23 | 5 | 3.69 |
| S172765 | 10 | 17,120,812 | 34.9 | 7 | 23 | 7 | 2.19 | 7 | 23 | 5 | 3.69 |
| S175248 | 10 | 20,145,999 | 34.9 | 6 | 24 | 7 | 3.32 | 7 | 23 | 5 | 3.69 |
| S175884 | 10 | 21,614,104 | 36.2 | 6 | 24 | 7 | 3.32 | 7 | 23 | 5 | 3.69 |
| S177499 | 10 | 25,155,121 | 36.2 | 6 | 18 | 8 | 0.75 | 7 | 22 | 4 | 4.21 |
| S180184 | 10 | 29,684,639 | 36.2 | 6 | 25 | 7 | 3.84 | 7 | 23 | 5 | 3.69 |
| S180589 | 10 | 30,366,529 | 36.2 | 6 | 23 | 7 | 2.83 | 7 | 23 | 5 | 3.69 |
| S182836 | 10 | 33,678,153 | 36.2 | 6 | 24 | 7 | 3.32 | 7 | 23 | 5 | 3.69 |
| S188391 | 10 | 42,433,003 | 36.9 | 6 | 25 | 7 | 3.84 | 7 | 21 | 5 | 2.70 |
| S190237 | 10 | 46,487,297 | 36.9 | 6 | 24 | 7 | 3.32 | 7 | 22 | 5 | 3.18 |
| S194142 | 10 | 57,447,381 | 43.7 | 7 | 28 | 4 | 7.87* | 8 | 19 | 7 | 0.53 |
| S194704 | 10 | 60,400,715 | 54.7 | 12 | 21 | 3 | 5.50 | 4 | 22 | 8 | 3.88 |
| S194933 | 10 | 61,851,949 | 65.1 | 19 | 18 | 1 | 17.16* | 2 | 22 | 10 | 6.71* |
| S195109 | 10 | 63,024,556 | 79.8 | 26 | 12 | 1 | 37.82* | 1 | 17 | 16 | 13.24* |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S195403 | 10 | 63,728,599 | 90.7 | 34 | 5 | 0 | 80.85* | 1 | 15 | 19 | 19.23* |
| S195527 | 10 | 63,892,947 | 93.5 | 36 | 3 | 0 | 94.38* | 1 | 13 | 20 | 23.12* |
| S195717 | 10 | 64,408,029 | 117.1 | 27 | 8 | 3 | 43.05* | 0 | 0 | 34 | 102* |
| S196053 | 11 | 576,180 | 0.0 | 17 | 15 | 7 | 7.21* | 6 | 19 | 10 | 1.17 |
| S196265 | 11 | 1,933,165 | 5.1 | 14 | 18 | 7 | 2.74 | 8 | 17 | 10 | 0.26 |
| S196497 | 11 | 3,614,670 | 17.3 | 11 | 17 | 10 | 0.47 | 5 | 18 | 11 | 2.24 |
| S196989 | 11 | 4,554,198 | 34.8 | 9 | 9 | 18 | 13.5* | 5 | 12 | 17 | 11.41* |
| S198825 | 11 | 8,435,423 | 47.0 | 7 | 13 | 14 | 4.76 | 10 | 7 | 16 | 13.12* |
| S203849 | 11 | 16,463,087 | 64.7 | 5 | 24 | 8 | 3.76 | 10 | 13 | 11 | 1.94 |
| S206001 | 11 | 20,484,488 | 65.7 | 5 | 23 | 9 | 3.05 | 9 | 13 | 11 | 1.73 |
| S208664 | 11 | 24,365,669 | 67.6 | 9 | 20 | 8 | 0.30 | 10 | 11 | 11 | 3.19 |
| S210881 | 11 | 28,404,727 | 72.9 | 6 | 17 | 6 | 0.86 | 3 | 13 | 9 | 2.92 |
| S213151 | 11 | 29,925,733 | 76.6 | 2 | 27 | 9 | 9.32* | 5 | 18 | 11 | 2.24 |
| S214214 | 11 | 32,112,889 | 81.0 | 4 | 27 | 8 | 6.59* | 8 | 15 | 11 | 1.00 |
| S217066 | 11 | 36,215,708 | 81.0 | 4 | 27 | 8 | 6.59* | 8 | 15 | 11 | 1.00 |
| S219423 | 11 | 39,178,924 | 81.7 | 4 | 22 | 6 | 4.75 | 9 | 14 | 11 | 1.29 |
| S220069 | 11 | 39,899,181 | 81.7 | 4 | 25 | 8 | 5.43 | 8 | 14 | 11 | 1.30 |
| S224416 | 11 | 44,684,531 | 81.7 | 1 | 22 | 8 | 8.61* | 8 | 11 | 11 | 2.73 |
| S228219 | 11 | 48,922,550 | 84.3 | 5 | 25 | 8 | 4.26 | 8 | 16 | 11 | 0.77 |
| S228995 | 11 | 50,179,319 | 85.2 | 6 | 24 | 8 | 2.84 | 8 | 16 | 11 | 0.77 |
| S229125 | 11 | 50,349,446 | 86.0 | 5 | 26 | 7 | 5.37 | 8 | 13 | 11 | 1.69 |
| S229272 | 11 | 50,560,514 | 88.4 | 7 | 17 | 6 | 0.60 | 10 | 8 | 10 | 5.14 |
| S229536 | 11 | 51,516,133 | 91.6 | 8 | 22 | 8 | 0.95 | 10 | 13 | 11 | 1.94 |
| S229768 | 11 | 52,132,900 | 96.7 | 6 | 24 | 8 | 2.84 | 12 | 14 | 9 | 1.91 |
| S229839 | 11 | 52,532,602 | 96.7 | 5 | 25 | 8 | 4.26 | 11 | 14 | 9 | 1.29 |
| S230640 | 12 | 209,922 | 0.0 | 7 | 23 | 9 | 1.46 | 6 | 11 | 13 | 5.40 |
| S230946 | 12 | 1,317,080 | 26.1 | 8 | 22 | 8 | 0.95 | 8 | 20 | 7 | 0.77 |
| S231052 | 12 | 1,811,385 | 30.8 | 8 | 24 | 6 | 2.84 | 8 | 18 | 5 | 1.39 |
| S231692 | 12 | 5,803,725 | 61.5 | 11 | 18 | 9 | 0.32 | 7 | 15 | 12 | 1.94 |
| S231878 | 12 | 7,165,691 | 65.9 | 9 | 16 | 9 | 0.12 | 8 | 14 | 13 | 2.83 |
| S232145 | 12 | 8,291,475 | 65.9 | 12 | 17 | 9 | 0.89 | 8 | 14 | 13 | 2.83 |
| S232394 | 12 | 9,128,273 | 71.8 | 10 | 21 | 8 | 0.44 | 7 | 14 | 10 | 0.87 |
| S233471 | 12 | 15,230,552 | 72.6 | 9 | 21 | 7 | 0.89 | 8 | 14 | 10 | 0.75 |
| S234301 | 12 | 18,148,304 | 83.3 | 14 | 12 | 4 | 7.87* | 10 | 6 | 6 | 6.00* |
| S235585 | 12 | 21,807,585 | 83.3 | 13 | 15 | 8 | 2.39 | 8 | 10 | 10 | 2.57 |
| S236203 | 12 | 24,071,628 | 84.8 | 15 | 14 | 9 | 4.53 | 13 | 8 | 10 | 7.84* |
| S238602 | 12 | 33,821,854 | 93.6 | 10 | 21 | 8 | 0.44 | 8 | 13 | 11 | 1.69 |
| S239025 | 12 | 35,762,126 | 94.3 | 9 | 20 | 8 | 0.30 | 7 | 15 | 13 | 2.77 |
| S239297 | 12 | 37,118,598 | 95.0 | 9 | 15 | 7 | 0.29 | 6 | 14 | 9 | 0.66 |
| S240093 | 12 | 40,454,923 | 95.0 | 10 | 21 | 8 | 0.44 | 7 | 15 | 10 | 0.69 |
| S241751 | 12 | 45,894,222 | 95.0 | 8 | 20 | 8 | 0.44 | 7 | 15 | 12 | 1.94 |
| S242837 | 12 | 51,478,012 | 96.4 | 9 | 19 | 8 | 0.17 | 8 | 13 | 13 | 3.35 |
| S243685 | 12 | 55,446,113 | 96.4 | 9 | 21 | 8 | 0.47 | 8 | 13 | 13 | 3.35 |
| S244678 | 12 | 58,138,136 | 97.9 | 9 | 16 | 8 | 0.09 | 8 | 13 | 13 | 3.35 |
| S245602 | 12 | 62,935,373 | 120.9 | 9 | 24 | 5 | 3.47 | 9 | 18 | 7 | 0.35 |
| S245641 | 12 | 63,371,069 | 129.7 | 8 | 25 | 6 | 3.31 | 9 | 18 | 8 | 0.09 |
| S245782 | 12 | 64,109,986 | 137.3 | 12 | 23 | 4 | 4.54 | 11 | 16 | 8 | 0.77 |
| S245979 | 12 | 65,139,260 | 144.2 | 10 | 23 | 5 | 3.00 | 9 | 19 | 6 | 1.00 |

*Significant at P ≤0.05

**Trait-based analysis (TBA)**

Trait-based analysis was employed to identify SNP markers associated with LB resistance loci. For any SNP marker, when PI 163245 allele frequency differences ($p_R$-$p_S$) between the resistant and susceptible classes were significant (i.e., $\geq 2\sigma_p$) it indicated that the PI 163245 genotype was associated with LB resistance at ≥95% confidence. Similarly, when the Fla. 8059 allele frequency differences between the two classes ($q_R$ - $q_S$) were significant, they indicated association of Fla. 8059 genotypes with LB resistance. Four genomic regions in PI 163245, located on chromosomes 2, 3, 10, and 11, were significantly associated with LB resistance (Table 4-5). On chromosome 2, the allele frequency differences for the first ten markers were skewed towards PI 163245, corresponding to a 25.5 cM region between markers S041728 and S055792. In the resistant class, the PI 163245 allele frequency was between 0.47 and 0.58. In contrast, within the susceptible class, the PI 163245 allele frequency at each locus was between 0.25 and 0.37 (Table 4-5). On this chromosome, a 2.8 cM region between markers S048832 and S054853 was associated with LB resistance at $>3\sigma_p$, corresponding to a genetic distance of 2.8 cM and a physical distance of approximately 17.7 Mbp (Table 4-5).

A single marker, S073679, on chromosome 3 was significantly associated with LB resistance. The allele frequency differences for the two flanking markers were also elevated, although not significantly. The genomic interval was delineated to a genetic distance of <11.7 cM, corresponding to a physical distance of 2.2 Mbp. The PI 163245 allele frequency in the resistant class at the S073679 locus was 0.57, while that in the susceptible class was 0.39 (Table 4-5).

Six markers on the bottom of chromosome 10, between S194704 and S195717 and corresponding to a 62.4 cM region, were significantly associated with LB resistance. The final five markers on chromosome 10 were associated with LB resistance at $>3\sigma_p$ and encompassed a 52.0 cM region, corresponding to a physical distance of just 2.6 Mbp. For these markers, the allele frequency differences between the resistant and susceptible classes were extreme, increasing from 0.18 to 0.82. Interestingly, the susceptible class contained no alleles conferred by PI 163245 for the markers most distally located on this chromosome, while in the resistant class the PI 163245 allele frequency for these markers was 0.82. While the genetic distance associated with resistance was large, the physical distance corresponded to just over 4.0 Mbp, although an additional 1.1 Mbp extends beyond the last marker on chromosome 10 and no markers were genotyped within this interval (Table 4-5).

Late blight resistance was detected for a single marker (S196053) at the top of chromosome 11. The allele frequency difference of the flanking marker, S196265, was also slightly elevated. The genomic region significantly associated with resistance occurs over an interval of <2 Mbp. In the resistant class, the PI 163245 allele frequency was 0.63, while that in the susceptible class was 0.44 (Table 4-5).

**Table 4-5** Loci significantly associated with late blight (LB) resistance. PI 163245 allele frequency differences were calculated between the resistant ($p_R$) and susceptible ($p_S$) classes and compared to the standard error of the marker allele frequency differences ($\sigma_p$). Allele frequency differences $\geq 2\sigma_p$ are considered associated with LB resistance at >95% confidence.

| Marker | Chromosome | Physical Locus (bp) | Genetic Locus (cM) | $p_R$ | $p_S$ | $p_R$-$p_S$ | $\sigma_p$ |
|---|---|---|---|---|---|---|---|
| S041728 | 2 | 537,665 | 0.0 | 0.47 | 0.25 | 0.22* | 0.08 |
| S043233 | 2 | 3,416,012 | 0.0 | 0.47 | 0.31 | 0.17* | 0.08 |
| S044782 | 2 | 6,233,893 | 6.7 | 0.57 | 0.33 | 0.23* | 0.08 |
| S045548 | 2 | 7,421,251 | 6.7 | 0.56 | 0.34 | 0.22* | 0.08 |
| S048832 | 2 | 15,226,512 | 8.1 | 0.56 | 0.30 | 0.26** | 0.08 |
| S053579 | 2 | 25,809,518 | 8.1 | 0.58 | 0.33 | 0.24** | 0.08 |
| S054749 | 2 | 31,192,221 | 9.5 | 0.55 | 0.30 | 0.26** | 0.08 |
| S054853 | 2 | 32,920,145 | 10.9 | 0.57 | 0.32 | 0.24** | 0.08 |
| S055431 | 2 | 34,968,684 | 16.4 | 0.52 | 0.34 | 0.17 | 0.09 |
| S055792 | 2 | 36,645,658 | 25.5 | 0.54 | 0.37 | 0.17* | 0.08 |
| S055963 | 2 | 37,415,171 | 28.3 | 0.51 | 0.40 | 0.11 | 0.08 |
| S073624 | 3 | 62,874,374 | 76.1 | 0.55 | 0.40 | 0.15 | 0.09 |
| S073679 | 3 | 63,035,518 | 78.4 | 0.57 | 0.39 | 0.18* | 0.08 |
| S074168 | 3 | 65,027,754 | 87.8 | 0.51 | 0.37 | 0.15 | 0.08 |
| S194142 | 10 | 57,447,381 | 43.7 | 0.54 | 0.51 | 0.02 | 0.08 |
| S194704 | 10 | 60,400,715 | 54.7 | 0.63 | 0.44 | 0.18* | 0.08 |
| S194933 | 10 | 61,851,949 | 65.1 | 0.74 | 0.38 | 0.35** | 0.08 |
| S195109 | 10 | 63,024,556 | 79.8 | 0.82 | 0.28 | 0.54** | 0.07 |
| S195403 | 10 | 63,728,599 | 90.7 | 0.94 | 0.24 | 0.69** | 0.06 |
| S195527 | 10 | 63,892,947 | 93.5 | 0.96 | 0.22 | 0.74** | 0.05 |
| S195717 | 10 | 64,408,029 | 117.1 | 0.82 | 0.00 | 0.82** | 0.04 |
| S196053 | 11 | 576,180 | 0.0 | 0.63 | 0.44 | 0.19* | 0.08 |
| S196265 | 11 | 1,933,165 | 5.1 | 0.59 | 0.47 | 0.12 | 0.08 |

*Marker allele frequency difference $\geq 2\sigma_p$
**Marker allele frequency difference $\geq 3\sigma_p$

Four regions on chromosomes 4, 7, 9, and 10 included markers at which the Fla. 8059 genotype significantly contributed to LB resistance. Five markers on chromosome 4, from S088532-S089706, were significantly associated with LB resistance conferred by Fla.8059. This region corresponded to a genetic distance of 22.3 cM and a physical distance of 6.7 Mbp. Fla. 8059 allele frequencies in the resistant class were slightly higher than expected based on Mendelian inheritance, ranging from 0.51-0.58, and substantially lower than expected in the susceptible class, ranging from 0.35-0.38 (Table 4-6).

One marker on chromosome 7, S133732, was also associated with LB resistance. Located near the bottom of the chromosome, the Fla. 8059 genotype was more highly represented in both the resistant and susceptible classes. The Fla. 8059 allelic frequency was 0.75 in the resistant class and 0.59 in the susceptible class (Table 4-6). The allele frequency difference was only elevated for one of the flanking markers.

Chromosome 9 contained the largest number of markers that favored Fla. 8059 in the resistant class. A total of four markers near the top of chromosome 9 over a 32.2 cM region (6.4 Mbp) were significantly associated with resistance. The allele frequency difference at three of the four markers was $>3\sigma_p$. In the resistant class, the Fla. 8059 allele frequency was as high as 0.75, while in the susceptible class the frequency fell as low as 0.37 (Table 4-6).

The marker S164390, near the top of chromosome 10, was also associated with LB resistance. The interval associated with this resistance was <8.1 cM and corresponded to a physical distance of <1.7 Mbp. The allele frequency difference was 0.18 and the Fla. 8059 allele frequency in the resistant class was slightly elevated at 0.55. However, in the susceptible class, the Fla. 8059 allelic frequency was 0.37.

**Table 4-6** Fla. 8059 allele frequency in resistant ($q_R$) and susceptible ($q_S$) classes at four genomic regions associated with late blight (LB) resistance. Allele frequency differences were calculated and compared to the standard errors of the allele frequency differences ($\sigma_p$). Allele frequency differences $\geq 2\sigma_p$ are considered associated with LB resistance at >95% confidence.

| Marker | Chromosome | Physical Locus (bp) | Genetic Locus (cM) | $q_R$ | $q_S$ | $q_R$-$q_S$ | $\sigma_p$ |
|---|---|---|---|---|---|---|---|
| S087073 | 4 | 48,328,855 | 16.6 | 0.55 | 0.44 | 0.11 | 0.08 |
| S088532 | 4 | 55,822,031 | 39.0 | 0.56 | 0.36 | 0.20* | 0.08 |
| S088929 | 4 | 59,296,245 | 44.0 | 0.58 | 0.38 | 0.20* | 0.08 |
| S089222 | 4 | 60,319,022 | 48.8 | 0.51 | 0.35 | 0.16* | 0.08 |
| S089706 | 4 | 62,522,878 | 61.3 | 0.57 | 0.36 | 0.21* | 0.08 |
| S090355 | 4 | 64,939,052 | 79.3 | 0.55 | 0.41 | 0.14 | 0.08 |
| S133230 | 7 | 61,211,852 | 74.9 | 0.55 | 0.53 | 0.02 | 0.09 |
| S133732 | 7 | 65,251,571 | 101.8 | 0.75 | 0.59 | 0.16* | 0.08 |
| S133903 | 7 | 66,584,336 | 108.8 | 0.72 | 0.58 | 0.14 | 0.08 |
| S148619 | 9 | 1,489,519 | 0.0 | 0.65 | 0.37 | 0.28** | 0.08 |
| S148817 | 9 | 2,136,373 | 7.0 | 0.75 | 0.38 | 0.37** | 0.08 |
| S149233 | 9 | 3,645,130 | 15.0 | 0.67 | 0.39 | 0.28** | 0.08 |
| S149840 | 9 | 6,390,031 | 32.2 | 0.62 | 0.46 | 0.16* | 0.08 |
| S150390 | 9 | 9,102,976 | 41.8 | 0.53 | 0.49 | 0.04 | 0.08 |
| S164390 | 10 | 41,931 | 0.0 | 0.55 | 0.37 | 0.18* | 0.08 |
| S164795 | 10 | 1,754,215 | 8.1 | 0.57 | 0.47 | 0.10 | 0.08 |

*Marker allele frequency difference $\geq 2\sigma p$
**Marker allele frequency difference $\geq 3\sigma p$

## Discussion

PI 163245 was highly resistant to LB in all experiments, and not statistically different from controls containing either *Ph-2* or *Ph-3* alone. However, PI 163245 was not as resistant as control lines containing *Ph-2* and *Ph-3* combined. In contrast, Fla. 8059 was highly susceptible to LB in all experiments and comparable to LB susceptible control lines. The response to LB infection in the $F_1$ progeny individuals was similar to but slightly lower than the mid-parental value, suggesting resistance conferred by PI 163245 is under co-dominant gene action (Table 4-1).

The $F_2$ population (n = 560) ranged from 0-100% DS and averaged 51.9% DS. The $F_2$ distribution (Fig. 4-1) was non-normal based on the Shapiro-Wilk test for normality ($P<0.001$). The mapping population was skewed slightly towards susceptibility (skewness = -0.194), indicating the resistance gene(s) are likely co-dominant or slightly

recessive, which was also supported by the high number of $F_2$ individuals with intermediate % DS and the moderate % DS exhibited by the heterozygous $F_1$ progeny generation.

The selected resistant (n = 39) and susceptible (n = 35) $F_2$ individuals averaged 7.5% and 90.4 % DS, respectively (Table 4-1). The resistant and susceptible $F_2$ phenotypes were confirmed in $F_3$ progeny families. Only $F_2$ parents of $F_3$ progeny families with minimal segregation were genotyped and included in QTL analysis. The averages for the $F_3$ progeny families, 18.0% and 86.0% DS for the resistant and susceptible classes, respectively, were slightly less extreme than those of their $F_2$ selected parents (7.5% and 90.4 % DS). This could be due to various reasons, including less than perfect heritability of the trait, differences in disease pressure in the $F_2$ parental and $F_3$ progeny generations, presence of non-additive gene actions, and segregation of minor resistance genes (Table 4-1, Fig. 4-2). However, based on these observed values, it is expected that most major resistance genes would be detected.

Previously, identification of sufficient polymorphic markers between *S. lycopersicum* and *S. pimpinellifolium* was often a challenging issue due to their close genomic relationship. However, in the present study, sequencing RRLs of PI 163245 and Fla. 8059 and comparison of the two genomes identified thousands of polymorphic SNPs suitable for genetic mapping. In total, 33,385 SNPs were identified (Table 4-2). Although not all SNPs were suitable for marker development, 70.0% of the selected SNPs for marker development were successfully converted to KASP markers. Additionally, genotyping of both parents validated 98.5% of markers, providing good confidence in this SNP identification protocol. The availability of these thousands of SNPs is expected

to be useful when developing additional markers for fine mapping. However, in the present study, only 233 were used for $F_2$ mapping and TBA.

A genetic map was developed based on the 74 $F_2$ individuals and 233 SNP markers (Table 4-3, Fig. 4-3). The order of the markers used for developing the genetic map corresponded perfectly with the physical map. The average genetic distance between SNP markers was 5.5 cM. The total genome length was 1,278.2 cM, which is fairly consistent with previously developed genetic maps derived from *S. lycopersicum* and *S. pimpinellifolium* (GRANDILLO AND TANKSLEY 1996; SHARMA *et al.* 2008; ASHRAFI *et al.* 2009; MERK *et al.* 2012; SIM *et al.* 2012). Although the genetic and physical distances between markers were generally small, there were several larger genetic gaps between markers resulting from high levels of recombination. However, no gaps larger than 33.3 cM were found and distances >20 cM corresponded to physical lengths of 7.4 Mbp or less (Table 4-4).

Due to the use of a large $F_2$ population (n = 560) and the intense bidirectional selections made, it is highly unlikely that any QTL with large effects was undetected. Navabi *et al.* (2009) reported that genotyping of just 14% of individuals from a population of 500 would be sufficient to detect a QTL explaining as little as 9% of the phenotypic variation, as long as the distance between the markers and resistance genes was ≤15 cM. In the present study, 14% of 560 $F_2$ individuals were selected and genotyped, and the largest theoretical distance between a genetic marker and resistance locus was 16.7 cM, suggesting that all QTLs of moderate or larger effects were identified.

In order to test for deviation from the expected Mendelian ratio (1:2:1), Chi-square analyses were performed at each locus separately for resistant and susceptible classes. In total, 56 (24%) markers distributed across nine chromosomes exhibited skewed segregation (Table 4-4). Nine SNPs deviated from the expected genotypic ratio of 1:2:1 in both the resistant and susceptible classes, seven of which were associated with LB resistance (Tables 4-4, 4-5, 4-6). The remaining two markers, which showed deviation from normal segregation in both classes, were each skewed in the same direction for both classes. One marker was skewed towards Fla. 8059 and the other towards PI 163245. Nearly half of the 56 abnormally segregating markers (41%) were associated with LB resistance (discussed below). For four genomic regions, the heterozygous class was overrepresented in the resistant or susceptible class. This could be potentially explained by heterozygous advantage for the two genomic intervals identified in the resistant class. Interestingly, both genomic regions with significantly higher levels of heterozygosity in the susceptible class were flanking regions significantly associated with LB resistance, suggesting these deviations could have been caused by phenotypic selection for susceptibility. The remaining markers only exhibited skewed segregation in one class favoring either the Fla. 8059 or PI 163245 genotype. Skewed segregation has often been reported in tomato for populations derived from interspecific crosses (GRANDILLO AND TANKSLEY 1996; CHEN AND FOOLAD 1999; LIPPMAN AND TANKSLEY 2001; ZHANG *et al.* 2003; SHARMA *et al.* 2008; ASHRAFI *et al.* 2009). Besides conscious phenotypic selection, segregation of genotypes deviating from expected ratios can be caused by self-incompatibility, unilateral incongruity, gametophytic selection, or viability selection (FOOLAD 1996; ASHRAFI *et al.* 2009). An advantage of bidirectional selective

genotyping is that skewed segregation from factors other than phenotypic selection for LB resistance is unlikely to affect QTL detection.

Trait-based analysis detected genomic regions on chromosomes 2, 3, 10, and 11 that were significantly associated with LB resistance conferred by PI 163245. The resistance associated with chromosomes 2 and 10 had the largest effects, and contained several loci with allele frequency differences $>3\sigma_p$, spanning genomic intervals of 25.5 cM and 62.4 cM, respectively. Each of chromosomes 3 and 11 had only a single marker significantly associated with resistance, suggesting their phenotypic effects may not have been as large.

Late blight resistance at the top of chromosome 2 encompassed a 25.5 cM (36.6 Mbp) interval (Table 4-5). Based on the tomato genome annotation (ITAG2.40, WWW.SOLGENOMICS.NET), this region contains 928 genes and 21 defense-related proteins or protein fragments including three genes encoding CC-NBS-LRR class proteins. Ten markers were significantly associated with LB resistance at $>2\sigma_p$, and four sequential markers from 8.1-10.9 cM (15.2-32.9 Mbp) were significant at $\geq 3\sigma_p$. Two LB resistance QTLs on the short arm of chromosome 2 had been previously reported in tomato. Brouwer *et al*. (2004), identified quantitative LB resistance associated with the cultivated tomato breeding line, NC 84173, across several experiments for a cross between *S. lycopersicum* and *S. habrochaites* and used two isolates of *P. infestans* from clonal lineages US-6 and US-11. The genomic region associated with this resistance, *lb2a*, was mapped between 10 and 54 cM. The QTL explained as much as 24% of the phenotypic variation (BROUWER *et al.* 2004). However, in the present study, based on the inclusion of NC 84173 in all screening experiments as a LB susceptible control and its high levels

of susceptibility to the US-23 isolate utilized (Table 4-1), it does not seem likely that *lb2a* is responsible for the resistance detected in PI 163245. The second LB resistance QTL identified on chromosome 2 was found in the *S. pimpinellifolium* accession L3708 and was denoted as *qPh2.1* (CHEN *et al.* 2014). This resistance QTL, was located at approximately 13.7 cM and is partially dominant. *qPh2.1* conferred resistance against the US-11 *P. infestans* isolate Pi733. It is unknown whether the QTL identified by Chen *et al*. corresponds to the resistance identified in PI 163245. Development of near-isogenic lines (NILs), fine mapping, and potential cloning of the gene are required in order to determine whether this resistance gene is the same as the previously reported *qPh2.1*, a different allele of *qPh2.1*, or a completely novel resistance gene. However, LB resistance on chromosome 2 has not been previously utilized in developing LB resistant tomato cultivars, suggesting this resistance could have some value in breeding durable resistance to LB.

Resistance was also identified on chromosome 3 (Table 4-5). Only one marker, S073679, was significantly associated with resistance, located at 78.4 cM (63.0 Mbp). However, several flanking markers from 65.3-98.4 cM displayed elevated allele frequency differences. Previously, LB resistance on chromosome 3 was reported in *S. habrochaites* (BROUWER *et al.* 2004). However, the interval associated with the reported resistance was nearly 64 Mbp long, making it difficult to determine if the regions correspond to each other (BROUWER *et al.* 2004). Development of NILs is needed to determine the effect of this resistance, although based on the relatively small allele frequency differences, this region does not likely play a substantial role in resistance conferred by PI 163245.

Six markers on chromosome 10 displayed the highest allele frequency differences in this mapping population, encompassing a 62.4 cM region, although this interval totals only 4.0 Mbp. Five markers were significantly associated with LB resistance at $>3\sigma_p$ from 65.1-117.1 cM. Based on the high allele frequency differences between resistant and susceptible class genotypes, it appears that this interval has the largest effect on resistance within this mapping population. The interval contains 711 genes, including seven potential resistance genes, two of which encode CC-NBS-LRR class proteins. The region also contains a higher *R* gene density of 1.75 per Mbp compared to 0.73 *R* genes/Mbp on average for the entirety of chromosome 10. This genomic locus corresponds to the same interval as *Ph-2*, a previously reported LB resistance gene in tomato (GALLEGLY 1960; PEIRCE 1971; MOREAU *et al.* 1998). The new LB resistance gene, *Ph-5*, located on chromosome 10 as reported by Merk *et al*. (2012) also co-localizes with this locus. Since *Ph-2* and *Ph-5* have not been fine mapped or cloned, additional research is needed to determine how these resistance genes correspond with the resistance reported in PI 163245. However, based on the performance of PI 163245 under prolonged LB infection in field conditions, PI 163245 substantially outperforms lines containing only *Ph-2*, suggesting additional factors are likely contributing to resistance beyond previously reported resistance genes (FOOLAD *et al.* 2014, R.G. GARDNER, *pers. comm.*). Additionally, LB resistance genes can occur in clusters, providing the possibility that these genes are tightly linked (PARK *et al.* 2005; PARK *et al.* 2009).

A single locus near the top of chromosome 11 was significantly associated with resistance at $>2\sigma_p$ associated with an interval of <1.9 Mbp (Table 4-5). The top of

chromosome 11 has an elevated density of defense related genes, averaging nearly twice as many *R* genes/Mbp (2.1 *R* genes/Mbp) as the rest of the chromosome (1.1 *R* genes/Mbp). The region contains 268 genes, including four potential resistance genes encoding CC-NBS-LRR proteins. However, the allele frequency differences were not as substantial as on chromosomes 2 and 10, suggesting the phenotypic effect of this interval was not particularly high. Previously, Brouwer *et al*. (2004) reported two QTLs on chromosome 11 (*lb11a* and *lb11b*) associated with LB resistance in *S. habrochaites*. The QTL, *lb11a* is also located near the top of chromosome 11, however it is unknown if the same gene is responsible in both instances. Additional examination of this region is needed to determine its individual effect and value in tomato breeding.

In addition to the resistance mapped in PI 163245, TBA detected several regions on chromosomes 4, 7, 9, and 10 in Fla. 8059, which appeared to be associated with LB resistance (Table 4-6). However, it is suspected that these genomic intervals were likely detected as a result of superior horticultural characteristics of Fla. 8059 rather than host resistance to LB. This is partially supported by few annotated defense related genes within these regions. In fact, chromosomes 4 and 10 do not appear to contain any CC-NBS-LRR encoding genes within these regions, while chromosomes 7 and 9 each contained just one. Additionally, Fla. 8059 was highly susceptible in all experiments, averaging more than 90% DS, and suggesting that these regions are not useful for breeding resistance to LB (Table 4-1).

The high level of resistance reported in PI 163245 suggests that this accession has strong breeding potential. Three of the four genomic loci associated with resistance have not previously been utilized in tomato breeding. Additionally, it is unknown whether the

interval on chromosome 10 corresponds to *Ph-2*, or if this resistance is novel to this study. It has previously been reported that *Ph-2* is often overcome by particularly aggressive isolates of *P. infestans* and the mechanism of resistance merely slows the rate of disease progression (MOREAU *et al.* 1998; FOOLAD *et al.* 2008). However, based on greenhouse screenings, it appears that LB resistance conferred by PI 163245 is at least as strong as control lines containing *Ph-2*. Additional field experiments and observations suggested PI 163245 contains stronger resistance than cultivars containing *Ph-2* and even outperformed L3708, the source of *Ph-3* (FOOLAD *et al.* 2014, FOOLAD *et al.*, unpublished data). However, this does not preclude PI 163245 from containing *Ph-2*, as the overall resistance could be modified by the additional resistance genes reported in this study.

Previously, PI 163245 was screened against clonal lineages US-13 and US-14, and the level of disease resistance was comparable to the resistance reported in this study (Table 4-1) (FOOLAD *et al*. 2014). However, further screenings of PI 163245 against additional isolates of *P. infestans* are needed to determine the spectrum of LB resistance in this accession. To determine the value of each of the resistance genes on chromosomes 2, 3, 10, and 11, further research is needed. Isolation of each of these genomic regions in near-isogenic lines is required to estimate the individual phenotypic effects of each of these loci. Fine mapping of these genes and potential cloning is desirable for marker-assisted selection. Near-isogenic line development and breeding for LB resistance using PI 163245 is currently in progress at The Pennsylvania State University.

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# Chapter 5 Mapping of late blight resistance in the wild tomato accession PI 224710

## Abstract

Late blight (LB), caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most destructive plant diseases. The occurrence of more aggressive and fungicide resistant *P. infestans* clonal lineages have emphasized the importance of discovering and incorporating new sources of genetic LB resistance in tomato and potato breeding. Currently, commercially available LB resistant tomato cultivars only utilize LB resistance genes *Ph-2* and *Ph-3*, both of which have been overcome by particularly aggressive *P. infestans* isolates. Recently, the *Solanum pimpinellifolium* accession PI 224710 was identified as a strong source of highly heritable LB resistance in tomato. In this study, a trait-based analysis (TBA) approach (a.k.a. selective genotyping) was employed to identify and map LB resistance loci in an $F_2$ population (n = 599) derived from a cross between PI 224710 and a LB susceptible tomato breeding line (Fla. 8059). SNP markers were identified for mapping by construction and sequencing of reduced representation libraries (RRLs) and more than 20,000 SNPs were discovered. The $F_2$ mapping population was screened and the most LB resistant (n = 40) and LB susceptible (n = 40) individuals were selected and genotyped with 144 SNP markers. Four genomic regions associated with LB resistance were identified in PI 224710 on chromosomes 1, 2, 10, and 12. While the resistance interval on chromosome 10 co-localizes the previously identified LB resistance genes *Ph-2* and *Ph-5*, the remaining regions identified in this study have not been utilized in tomato breeding. Efforts are currently in progress to incorporate these LB resistance QTLs into tomato breeding lines.

## Introduction

Late blight (LB) is one of the most destructive diseases of tomato (*S. lycopersicum* L.) and potato (*S. tuberosum* L.) worldwide. Caused by the oomycete, *Phytophthora infestans* (Mont.) de Bary, LB affects all above ground parts of the plant as well as potato tubers. Late blight is notoriously difficult to control, often destroying susceptible crops within several days of infection. Late blight can spread extremely rapidly, due to the pathogen's short asexual lifecycle of fewer than five days and its ability to produce of as many as 300,000 sporangia/day from each lesion. Detecting low levels of the disease is often difficult and fungicide applications are frequently too late to salvage susceptible crops (FRY AND GOODWIN 1997). Entire tomato fields can be destroyed in 7-10 days (FOOLAD *et al.* 2008).

Accounting for the costs of fungicide control and yield loss, the economic impact of LB is enormous. The costs associated with fungicide treatments and lost yield in potato alone are estimated to exceed $6 billion annually. (HAVERKORT *et al.* 2008; HAVERKORT *et al.* 2009). In United States, nearly 7% of all tomato yield is lost to LB, and the disease has similar impacts on tomato production worldwide (NOWICKI *et al.* 2012). Identification and breeding for new genetic resistance to LB is an economically and environmentally friendly strategy for reducing the economic burden of the disease on growers and consumers. Late blight resistance has been studied extensively in potato and more than 30 LB resistance genes have been identified within wild potato species (HEIN *et al.* 2009; VLEESHOUWERS *et al.* 2011). Additionally, more than 20 of these potato LB resistance genes have been cloned, all of which encode CC-NBS-LRR class proteins

(VLEESHOUWERS *et al.* 2011; JO *et al.* 2015). However, LB resistance in commercial tomato cultivars is currently limited primarily to two LB resistance genes, *Ph-2* and *Ph-3*.

All major LB resistance genes successfully incorporated into commercial tomato cultivars were derived from the wild tomato species, *S. pimpinellifolium*. The first resistance gene, *Ph-1*, was discovered in West Virginia 19 and 731 and was mapped to chromosome 7 (BONDE AND MURPHY 1952; GALLEGLY AND MARVEL 1955; PEIRCE 1971). While several commercial cultivars were released containing this resistance gene, *Ph-1* is no longer considered valuable due to its ineffectiveness against the most common *P. infestans* race T-1 (FOOLAD *et al.* 2008). The second tomato LB resistance gene, *Ph-2*, was identified in West Virginia 700 (GALLEGLY AND MARVEL 1955). *Ph-2* is characterized by incomplete dominance and is often ineffective against particularly aggressive *P. infestans* isolates or under high disease pressure (GOODWIN *et al.* 1995; BLACK *et al.* 1996; FOOLAD *et al.* 2008). *Ph-2* was mapped to an 8.4 cM interval on the long arm of chromosome 10 (MOREAU *et al.* 1998). While *Ph-2* does not always confer particularly high levels of resistance individually, when combined with *Ph-3* high levels of LB resistance are obtained (GARDNER AND PANTHEE 2010b; GARDNER AND PANTHEE 2010a; PANTHEE AND GARDNER 2010). *Ph-3* was identified in L3708 (a.k.a LA 1269 and PI 365957) and was mapped to the long arm of chromosome 9 (CHUNWONGSE *et al.* 2002). *Ph-3* is currently the strongest single LB resistance gene available in commercial tomato cultivars and the only tomato LB resistance gene which has been fine mapped and cloned (ZHANG *et al.* 2013; ZHANG *et al.* 2014). Similarly to cloned LB resistance genes in potato, *Ph-3* encodes a CC-NBS-LRR protein (ZHANG *et al.* 2014).

In addition to the aforementioned genes, resistance has been identified in the *S. habrochaites* accessions LA 2099, BGH6902, and LA 1777 as well as the *S. pennellii* accession LA 716 (BROUWER *et al.* 2004; BROUWER AND ST. CLAIR 2004; ABREU *et al.* 2008; LI *et al.* 2011). Brouwer *et al*. (2004) identified quantitative LB resistance QTLs in LA 2099 distributed across all 12 chromosomes. The strongest QTLs were fine mapped on chromosomes 4, 5, and 11 (BROUWER AND ST. CLAIR 2004). However, due to their tight linkage with undesirable characteristics including poor canopy shape, small fruit size, and late maturity, these QTLs have so far been unusable in tomato breeding (BROUWER AND ST. CLAIR 2004). Mapping of LB resistance in LA 1777 identified five LB resistance QTLs, four of which co-localized with QTLs mapped in LA 2099. However, one novel QTL was discovered on chromosome 4 (LI *et al.* 2011). While the LB resistance conferred by BGH6902 has not been mapped, estimates of as many as 28 LB resistance genes and low heritability suggest this accession would be difficult to use for breeding LB resistance (ABREU *et al.* 2008). A QTL in LA 716 tightly linked to the self-pruning (*Sp*) locus on chromosome 6 accounted for close to 25% of the phenotypic variance for LB resistance within an $F_2$ mapping population (SMART *et al.* 2007). However, this QTL has not been used commercially.

Several additional LB resistance genes and resistant accessions have been reported within *S. pimpinellifolium*. Two LB resistance QTLs on chromosomes 1 and 10 were identified in PI 270443, a highly resistant *S. pimpinellifolium* accession with similar resistance as control lines containing *Ph-2* and *Ph-3* combined (MERK *et al.* 2012; MERK AND FOOLAD 2012; FOOLAD *et al.* 2014a; FOOLAD *et al.* 2014b). Additionally, the resistance was highly heritable and breeding efforts utilizing this accession have been

promising (MERK AND FOOLAD 2012; M.R. FOOLAD, *pers. commun.*). A second minor resistance QTL was mapped in L3708, the source of *Ph-3*, to the short arm of chromosome 2, though its usefulness in breeding is currently unknown (CHEN *et al.* 2014). LB resistance has also been identified in the *S. pimpinellifolium* accession L3707 and estimates suggest the involvement of two resistance loci (IRZHANSKY AND COHEN 2006). Evaluation of nearly 70 *S. pimpinellifolium* accessions in multiple field, greenhouse, and detached leaflet experiments identified 12 accessions (including PI 270443) with similar LB resistance as controls containing *Ph-2* and *Ph-3* combined (FOOLAD *et al.* 2014a; FOOLAD *et al.* 2014b). From these accessions, PI 224710 was selected for further genetic characterization and mapping of LB resistance.

Two estimates of LB heritability based on $F_2$:$F_3$ parent-offspring correlation analyses averaged 0.87, suggesting that the resistance conferred by PI 224710 is highly heritable (discussed in Chapter 3). Consequently genetic mapping and breeding efforts were initiated. Identifying genomic regions associated with LB resistance is valuable for breeders, as it facilitates marker-assisted selection (MAS) or transgenesis. Additionally, characterizing the genes responsible for resistance could help provide a broader understanding of LB resistance genes and potential mechanisms of *P. infestans* pathogenicity.

Two general approaches have been developed for mapping genes. The first approach is marker-based analysis (MBA), proposed by Thoday (1961). In this approach, the entire mapping population is phenotyped for one or more traits and all individuals are genotyped to identify associations between genetic markers and the trait(s) of interest (THODAY 1961). The Thoday method is desirable primarily when simultaneously

mapping multiple traits or when genotyping costs are lower than those incurred developing and phenotyping the population. The second approach is trait-based analysis (TBA; a.k.a. selective genotyping), first proposed by Stuber *et al.* (1980). In this approach, only individuals falling within the tails of the phenotypic distribution are genotyped (STUBER *et al.* 1980; LEBOWITZ *et al.* 1987; LANDER AND BOTSTEIN 1989). Trait-based analysis is generally more desirable than MBA when a single trait is being studied or when the expense of genotyping is substantially higher than the costs of phenotyping. TBA has equivalent power to MBA, while substantially reducing the required numbers of genotyped individuals at the expense of phenotyping a larger mapping population (DARVASI AND SOLLER 1992). TBA may be particularly useful for identifying QTLs with large phenotypic effects, which are generally more desirable for breeders (NAVABI *et al.* 2009). Since LB resistance was the only trait targeted for mapping in this study and phenotyping costs were low, a TBA approach was selected. While TBA can be performed unidirectionally (genotyping one extreme phenotypic class) or bidirectionally (genotyping both phenotypic classes), bidirectional analysis is at least as powerful as unidirectional analysis (NAVABI *et al.* 2009). Additionally, bidirectional analysis reduces the probability of false QTL detection resulting from skewed segregation, which can often occur in populations derived from interspecific crosses. Thus a bidirectional approach was selected.

To perform either MBA or TBA, large numbers of polymorphic genetic markers are required. Previously, identification of polymorphic genetic markers between *S. lycopersicum* and *S. pimpinellifolium* was often challenging due to high genomic similarity. However, the availability of the tomato reference genome, more affordable

sequencing costs, and the development of cost effective SNP genotyping methodologies have greatly facilitated marker development. Based on these factors, SNPs were identified for this study by sequencing and comparing parental reduced representation libraries (RRLs). Reduced representation libraries lessen genome complexity considerably by excluding highly repetitive and non-informative genomic regions during sequencing. Additionally, sequencing costs are reduced without sacrificing sequencing depth for SNP calling (ALTSHULER *et al.* 2000). While the overall coverage of the genome is somewhat diminished, for mapping in an $F_2$ population where linkage disequilibrium (LD) is high, the genome coverage is likely sufficient. Additionally, this SNP calling method has been successfully used in studies of human (ALTSHULER *et al.* 2000), soybean (HYTEN *et al.* 2010), sorghum (NELSON *et al.* 2011), and flax (KUMAR *et al.* 2012).

The objective of this study is to map LB resistance QTLs in the *S. pimpinellifolium* accession PI 224710. SNP markers were developed by sequencing RRLs and selective genotyping (TBA) was performed on resistant (n = 40) and susceptible (n = 40) class individuals in an $F_2$ (n = 599) mapping population to identify LB resistance QTLs in PI 224710.

## Materials and Methods

### Plant Material

A mapping population was developed through hybridization of the LB susceptible Fla. 8059 and the LB resistant accession PI 224710. Fla. 8059 is a tomato breeding line which produces firm fruit with high lycopene and good overall flavor (SCOTT *et al.* 2008). However, Fla. 8059 is not resistant to LB. Original seed of Fla. 8059 was provided

by J. W. Scott at the University of Florida, Gulf Coast Research and Education Center, Wimauma, FL, USA. PI 224710 is highly resistant to LB, but has indeterminate growth habit and small fruit. Seed of PI 224710 was obtained from the USDA Plant Genetics Resources Unit (PGRU), Geneva, NY, USA. The $F_1$ progeny were self-pollinated and a large $F_2$ mapping population consisting of 599 plants was grown and screened for LB resistance. The most resistant and susceptible $F_2$ individuals were selected and self-pollinated to produce $F_3$ progeny families. $F_3$ progeny families were grown and screened two separate times to confirm the resistant or susceptible phenotype of their $F_2$ parents. In each disease screening, parental lines, $F_1$ progeny, and LB resistant and susceptible control genotypes were included. Control lines consisted of NC 84173 (LB susceptible), New Yorker (*Ph-1*), NC 63EB (*Ph-2*), NC 870 (*Ph-3*), and NC 03220 (*Ph2 + Ph-3*). Seed for control lines was provided by R.G. Gardner at the North Carolina State University, Mills River, NC, USA.

**Inoculum preparation**

The US-23 *P. infestans* isolate RS2009T1 was used in all experiments. RS2009T1 was originally collected from a commercial tomato field at Rock Springs, PA and is race T-1, mating type A1. The inoculum was cultured on LB susceptible tomato leaflets placed abaxial (lower) side up in 10 × 150 mm Petri dishes. The lid of each Petri dish was lined with a layer of 1.7% water agar to maintain high humidity. The infected leaflets were incubated for 7-11 days until sufficient sporangia production occurred. Incubation conditions were 14-16 °C and 100% relative humidity (RH) on a 12 hour photoperiod. Following incubation, the leaflets were placed in 500 mL of chilled water and maintained at 4 °C for 1 hour to facilitate zoospore release. The solution was then briefly vortexed to

dislodge sporangia and the inoculum was filtered through cheesecloth to remove leaf debris. The solution was adjusted to 10,000 sporangia/mL using a haemocytometer and light microscope.

**$F_2$ and $F_3$ disease evaluations and selections**

A large $F_2$ mapping population (n = 599), parental lines, $F_1$ progeny, and resistant and susceptible control lines were grown in 72-cell flats in an environmentally controlled greenhouse. Parental, $F_1$, and control genotypes were grown in four replications consisting of six plants and placed on opposite sides and ends of the greenhouse compartment. Six weeks after planting, greenhouse conditions were adjusted to 16-18 °C, RH was increased to 95-100% using high pressure foggers, and blackout curtains were used to reduce ambient lighting in order to suppress hypersensitive and salicylic acid defense responses (GRIEBEL AND ZEIER 2008; RODEN AND INGLE 2009). Clear plastic was wrapped around each bench to prevent direct accumulation of water on the plants. After approximately six hours, the plants were gently misted with water. After 30 minutes, the inoculum was sprayed uniformly over all plants and a second application was applied 30 minutes later. Inoculum was applied at a volume of 1 L/1,000 plants and a concentration of 10,000 sporangia/mL. The following day, the blackout curtains were removed to allow ambient lighting for the remainder of the experiment.

Five days after inoculation, the plants were evaluated based on their percent disease severity (% DS) on a scale of 0-100%. A score of 0% indicated no symptoms and a score of 100% indicated no remaining healthy tissue or complete defoliation. Each $F_2$ plant was assigned an individual % DS score, while parental, $F_1$, and control replicates were assigned a % DS based on the overall average of the six plants. The most resistant

(% DS <10) and most susceptible but also surviving (% DS >85) $F_2$ individuals were identified, grown to maturity, and self-pollinated for $F_3$ seed to confirm the $F_2$ phenotype. Tissue was collected from all $F_2$ individuals and stored at -80 ºC for DNA extractions.

The $F_3$ progeny families were screened in two separate experiments (I and II). Disease screenings were conducted similarly to the $F_2$ experiment. However, each family was grown in two replicates in each experiment and placed separately in two similar greenhouse compartments to accommodate the larger population size. Experiment I was evaluated six days after inoculation and experiment II was evaluated five days after inoculation. The corresponding $F_2$ parents of $F_3$ progeny families averaging <20% DS (n = 40) and parents of susceptible class $F_3$ progeny families averaging >80% DS were selected for marker genotyping.

**Marker development**

A large number of polymorphic genetic markers were needed to map LB resistance. SNPs were identified between Fla. 8059 and PI 224710 by constructing, sequencing, and comparing reduced representation libraries (RRLs). The methods utilized were based on previously developed genotyping-by-sequencing (GBS) protocols (ELSHIRE *et al.* 2011). Briefly, DNA was extracted from Fla. 8059 and PI 224710 using the DNeasy Plant Mini Kit (Qiagen, Seoul, Korea) following the manufacturers protocol. The DNA was plated with adapter pairs and digested with the restriction enzyme *NlaIII*, which is a frequent cutter and methylation insensitive. The DNA was ligated to the barcoded adapters, pooled, and purified. Restriction fragments (RFs) were amplified and purified a second time. The fragment sizes were determined to ensure the majority of fragments fell between 170-350 bp. Paired-end sequencing was performed using an

Illumina Hi-seq 2000. Library construction and sequencing were performed by BGI@UC Davis.

The FASTQ sequencing output files were demultiplexed and mapped to the reference tomato genome sequence SL2.50 (WWW.SOLGENOMICS.NET). Reads were mapped using CLC Genomics Workbench (WWW.CLCBIO.COM). Single-end alignment was utilized instead of paired-end alignment due an issue with one adapter sequence. SNPs were called between PI 224710 and Fla. 8059 using SAMtools (LI *et al.* 2009). To ensure the quality of the SNP markers used in this study, requirements of at least three reads for each parent and no other mutations within 50 bp were implemented. A subset of SNPs (n = 373) was selected for marker development and validation.

**$F_2$ DNA extraction**

DNA was extracted from the selected $F_2$ genotypes using a modified quick extraction method (KING *et al.* 2014). $F_2$ tissue was collected and stored at -80 °C prior to disease screenings. Leaf tissue 2-3 cm in diameter was added to 160 µl NaCl (5 M) and 240 µl extraction buffer (200 mM Tris/HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) and pulverized using a Qiagen TissueLyser II or ground manually using a sterile plastic micropestle. After incubating for 30 minutes at 60 °C, the samples were centrifuged for 5 minutes at 2,500 × *g*. The supernatant was combined with an approximately equal volume of -20 °C isopropanol and inverted several times. The solution was incubated for 15 minutes at -20 °C and centrifuged for 5 minutes at the same speed. The samples were decanted and washed with 200 µl 70% ethanol and centrifuged for 5 minutes. The ethanol was decanted and the samples were dried at room temperature or placed in a drying oven at 60 °C until most of the ethanol had evaporated. The DNA

was resuspended in Tris/EDTA and quantified using a NanoDrop 2000 (THERMOSCIENTIFIC). Concentrations were adjusted to 30-60 ng/mL for KASP genotyping.

**Genotyping**

A total of 144 SNP markers which were distributed across the genome were selected for genotyping based on confirmed polymorphism, homozygosity within each parent, and the quality and consistency of fluorescent clustering for the homozygous and heterozygous genotypes. The 80 selected $F_2$ individuals were genotyped with all 144 markers using Kompetitive Allele Specific PCR (KASP) markers, and genetic mapping and trait-based QTL mapping were performed. KASP marker development and genotyping were performed at Ag-biotech, Monterey, CA, USA.

**Genetic map development**

To confirm the locus for each SNP and calculate genetic distances between markers, a linkage map was developed. The genetic map distances were calculated using MapMaker 3.0 (LANDER *et al.* 1987). The GROUP command was used to assign markers to a linkage groups and the ORDER command was used to determine the most likely SNP order. Marker orientation was verified using the RIPPLE command.

**Trait-based analysis (TBA) and other statistics**

A TBA approach was employed to identify markers associated with LB resistance. The marker allele frequency differences between the resistant and susceptible classes were calculated and compared to the standard error ($\sigma_p$) of the differences between the marker allele frequencies. The standard error ($\sigma_p$) was calculated using the following binomial equation:

$\sigma_p = (p_R q_R / 2N_R + p_S q_S / 2N_S)^{0.5}$,

where $p_R$ is the PI 224710 marker allele frequency in the resistant class, $p_S$ is PI 224710 marker allele frequency in the susceptible class, $q_R$ is the marker allele frequency of Fla. 8059 in the resistant class, $q_S$ is the marker allele frequency of Fla. 8059 in the susceptible class, and $N_R$ and $N_S$ are the numbers of individuals in the resistant and susceptible classes respectively. Allele frequency differences between the two selected classes of $>2\sigma_p$ were considered associated with resistance with at least 95% confidence (STEEL AND TORRIE 1980; LEBOWITZ *et al.* 1987).

Chi-square ($\chi^2$) goodness-of-fit tests were performed within each selected class to identify marker segregation which deviated from the expected Mendelian ratio of 1:2:1.

Mean comparisons of the parental, $F_1$, and resistant and susceptible control lines were calculated using Tukey's HSD test.

## Results

### Parental and control response to late blight infection

The average % DS of parental lines, $F_1$ progeny, and control genotypes are presented in Table 5-1. Fla. 8059 was highly susceptible to LB in all experiments and averaged nearly 90% DS. Fla. 8059 was not statistically different from the LB susceptible controls NC 84173 and New Yorker, which both averaged approximately 95% DS. In contrast, PI 224710 was highly resistant to LB, averaging <15% DS. PI 224710 was most similar to NC 870 (*Ph-3*) and was not statistically different from NC 03220 (*Ph-2* + *Ph-3*). The $F_1$ progeny were similar to PI 224710 and averaged 17.1% DS, suggesting that the LB resistance is under dominant control.

**Table 5-1** Response to late blight infection as a measure of percent disease severity (% DS) for parental lines, $F_1$, $F_2$, and $F_3$ generations and control genotypes.

| Genotype | Number of individuals or families | % DS[1] | Range (% DS) |
|---|---|---|---|
| $P_1$ (Fla. 8059) | 120 | 89.4 ± 10.8[a] | 60-100 |
| $P_2$ (PI 224710) | 120 | 13.9 ± 6[cd] | 5-25 |
| $F_1$ | 48 | 17.1 ± 5.6[bc] | 7-25 |
| NC 84173 | 96 | 95.4 ± 4.8[a] | 80-100 |
| New Yorker (*Ph-1*) | 120 | 94.1 ± 4.9[a] | 80-100 |
| NC 63EB (*Ph-2*) | 120 | 26.7 ± 18.7[b] | 5-70 |
| NC 870 (*Ph-3*) | 120 | 13.8 ± 6.9[cd] | 5-25 |
| NC 03220 (*Ph-2* + *Ph-3*) | 96 | 3.4 ± 1.8[d] | 0-7 |
| $F_2$ population | 599 | 52.5 ± 32.1 | 0-100 |
| $F_2$ susceptible class | 40 | 91.5 ± 3.5 | 85-97 |
| $F_2$ resistant class | 40 | 3.4 ± 2.2 | 0-8 |
| $F_3$ susceptible class families | 40 | 89.5 ± 14.8 | 10-100 |
| $F_3$ resistant class families | 40 | 9.7 ± 12.8 | 0-100 |

[1]Mean comparisons of parental and control lines were determined using Tukey's HSD test and are denoted by superscripts (a-d).

## $F_2$ and $F_3$ response to late blight infection

The distribution of the $F_2$ population (n = 599) was somewhat bimodal and ranged from 0-100% DS. The mean % DS was similar to the mid-parent value (51.7% DS), averaging 52.5% DS (Table 5-1, Fig. 5-1). The distribution was non-normal ($P < 0.001$) based on the Shapiro-Wilk test and skewed towards susceptibility (skewness = -0.25). Overall, slightly more than half the $F_2$ individuals (n = 308) averaged higher than the mean % DS (51.7% DS).

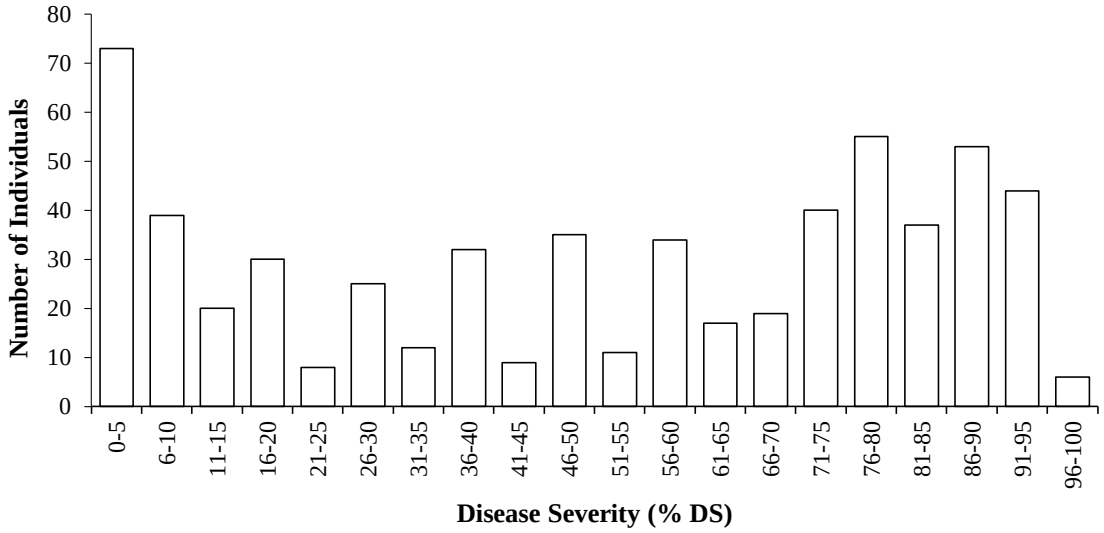



**Figure 5-1** Frequency distribution of disease severity (% DS) for an $F_2$ population (n = 599) derived from a cross between Fla. 8059 and PI 224710. Foliar % DS was measured as a percentage of whole plant foliar disease symptoms/defoliation on a scale of 0-100% DS.

The 80 most resistant and susceptible individuals were selected for genotyping. The resistant class $F_2$ individuals (n = 40) averaged <5% DS and ranged from 0-8% DS (Table 5-1). $F_3$ progeny testing in two experiments confirmed resistant class families generally resembled their $F_2$ parent. None of the selected resistant $F_3$ progeny families averaged >20% DS. The selected susceptible $F_2$ individuals were similar to Fla. 8059, averaging slightly higher than 90% DS (Table 5-1). The average susceptible class $F_3$ progeny family averaged 89.5% DS, confirming the susceptibility of their $F_2$ parents (Table 5-1, Fig. 5-2).

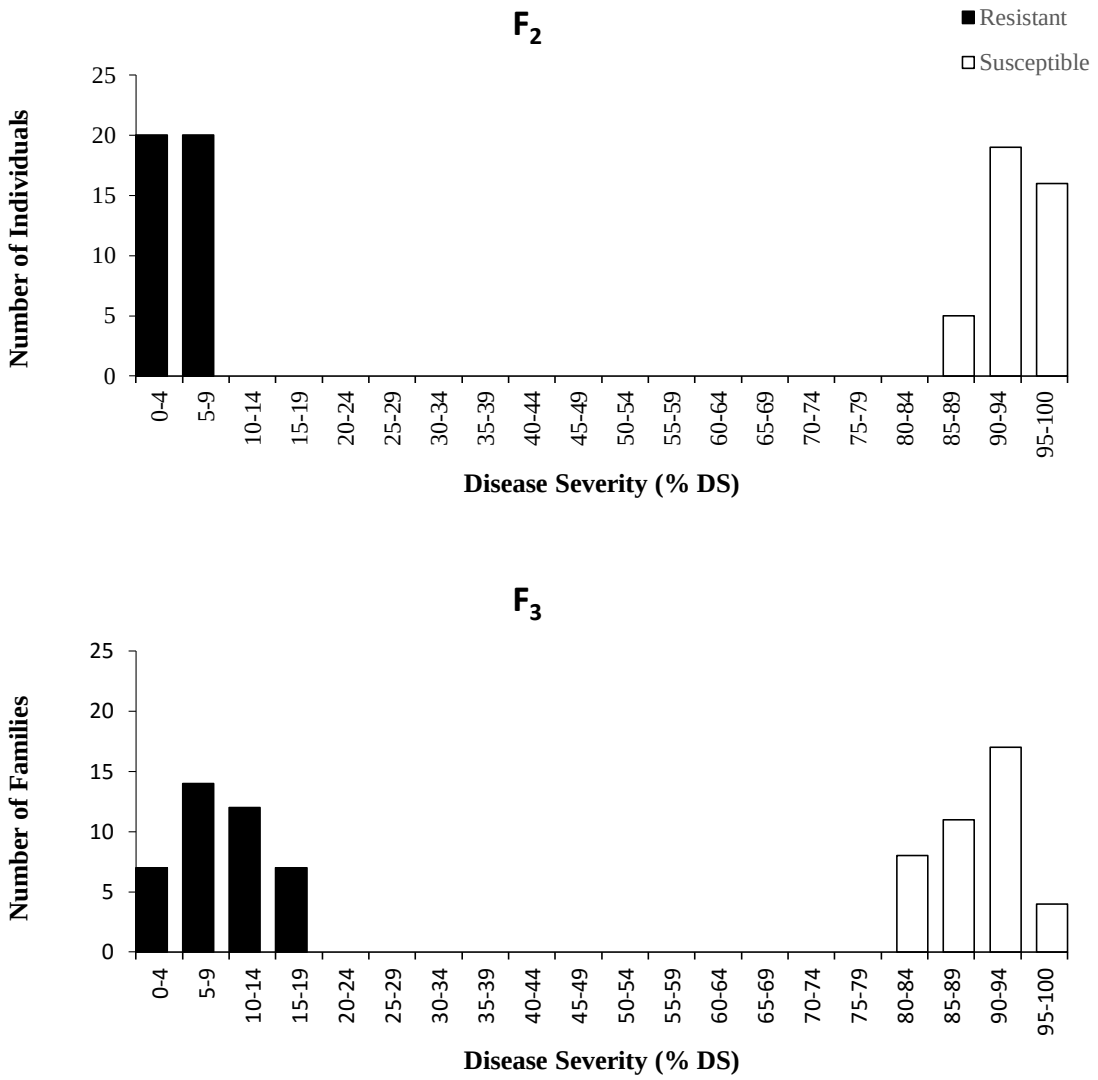



**Figure 5-2** Percent disease severity (% DS) distribution of genotyped $F_2$ individuals (top) and the mean % DS of their $F_3$ progeny families (bottom). Foliar disease severity was measured on a scale of 0-100%.

## Marker discovery and validation

Sequencing of the two parental RRLs generally produced uniform coverage with adequate read depth. The mapped Fla. 8059 RRL sequence covered 27% of the reference genome and the sequencing depth averaged 1.29. Excluding zero-coverage regions, the mean sequencing depth increased from 1.29 to 4.68. Genome coverage was fairly uniform, averaging 462 bp between reads, and no gaps exceeded more than 3.05 Mbp. In

total, 74.9% of the 11,285,819 reads were successfully mapped to the reference genome. The PI 224710 RRL sequence was fairly consistent, covering 19% of the genome with an average read depth of 0.52. Excluding zero-coverage regions, read depth averaged 2.67. The distance between reads was generally small, averaging 566.6 bp and the maximum distance between reads of 3.05 Mbp was similar to Fla. 8059. Overall, 4,562,318 reads were generated and 73.1% were successfully mapped to the reference genome.

More than 20,000 SNPs were identified between Fla. 8059 and PI 224710 (Table 5-2). Chromosome 11 contained the largest number of SNPs (4,531) and averaged just 12 kb between markers. The fewest polymorphic SNPs were located on chromosome 6, for which only 177 SNPs were identified. The majority of chromosomes averaged fewer than 100 kbp between markers, however chromosomes 6, 7, and 8 averaged 276 kbp, 124 kbp, and 338 kbp respectively.

**Table 5-2** SNPs between late blight (LB) susceptible Fla. 8059 and LB resistant PI 224710. SNPs were called based on at least three reads per genotype and no additional mutations within 50 bp of the given locus.

| Chromosome | Total SNPs | Average physical distance (bp) | Total physical distance (bp) |
|---|---|---|---|
| 1 | 1,262 | 76,279 | 96,263,686 |
| 2 | 852 | 64,363 | 54,837,473 |
| 3 | 2,286 | 30,849 | 70,519,770 |
| 4 | 3,053 | 21,708 | 66,275,631 |
| 5 | 1,771 | 37,077 | 65,662,558 |
| 6 | 177 | 275,837 | 48,823,175 |
| 7 | 541 | 124,308 | 67,250,533 |
| 8 | 195 | 337,522 | 65,816,723 |
| 9 | 914 | 79,101 | 72,298,755 |
| 10 | 2,948 | 22,218 | 65,499,759 |
| 11 | 4,631 | 12,151 | 56,273,154 |
| 12 | 2,264 | 29,316 | 66,371,490 |
| Total | 20,894 | - | 795,892,707 |

A total of 373 SNPs were selected for KASP marker development. However, only 144 markers were suitable for map construction. A total of 261 SNPs were successfully converted to reproducible KASP assays with consistent fluorescent clustering of the

homozygous and heterozygous genotypes. However, due to high levels of heterozygosity in PI 224710, only 144 SNPs were both homozygous and polymorphic between the PI 224710 and Fla. 8059 parents used for developing the mapping population.

**Genetic map construction**

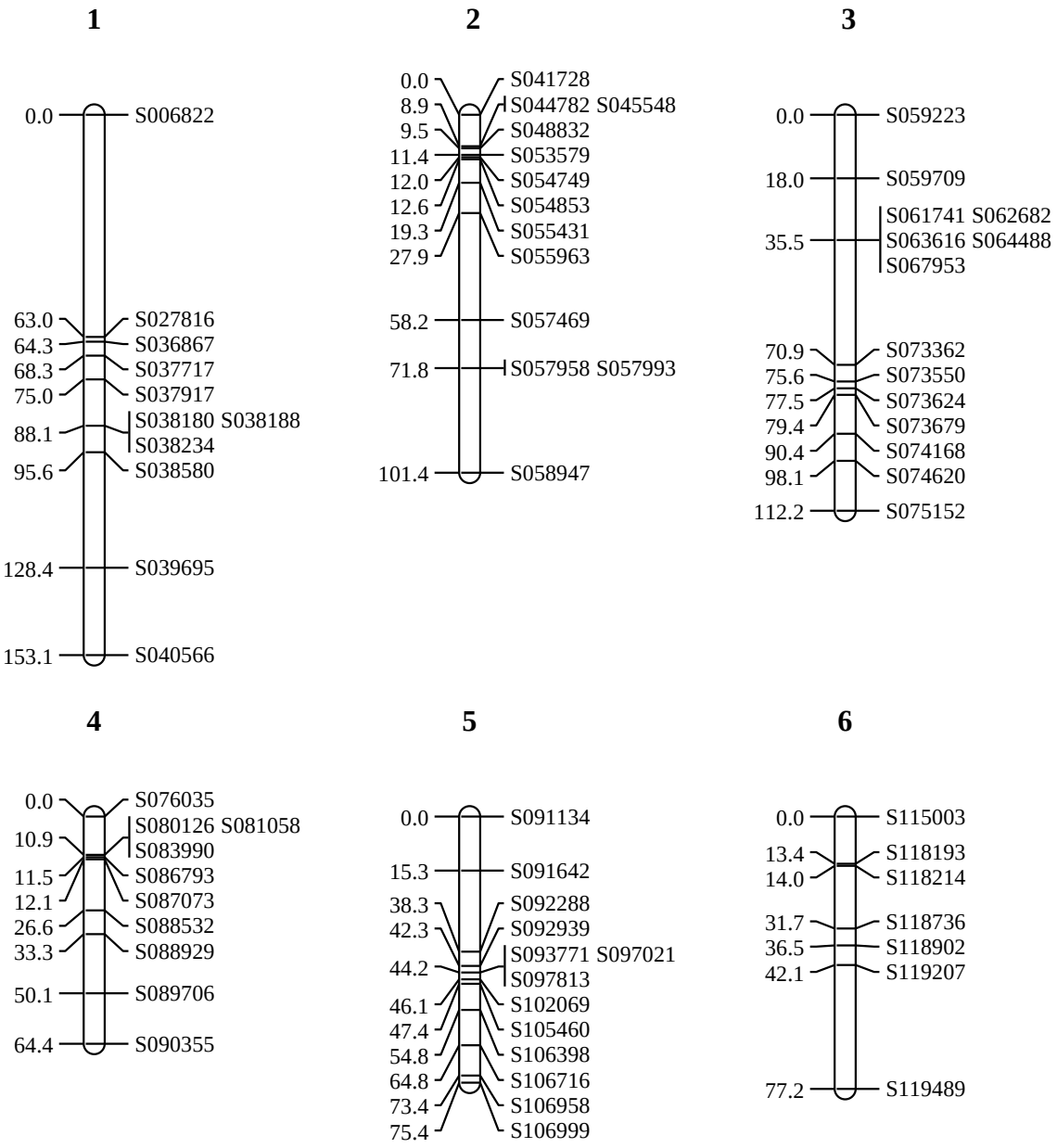
A genetic map based on 144 SNP markers was constructed based on a cross between Fla. 8059 and PI 224710. Genetic mapping generated 12 linkage groups corresponding to the 12 tomato chromosomes, which ranged from 72.3-153.1 cM in length (Table 5-3, Fig. 5-3). The number of markers assigned to each linkage group (chromosome) ranged from 7-17 SNPs and the average physical distance between markers was 5.6 Mbp. The mean genetic distance between markers ranged from 4.4-13.9 cM per chromosome and overall averaged 7.4 cM.

**Table 5-3** Genetic mapping of SNP markers (n = 144) in an $F_2$ mapping population (n = 80). Individuals were genotyped using KASP and a genetic map was developed using MapMaker 3.0 (LANDER *et al.* 1987).

| Chromosome | Chromosome Length (cM) | SNPs Genotyped | Average physical distance (bp) | Average Genetic distance (cM) |
|---|---|---|---|---|
| 1 | 153.1 | 11 | 9,302,149 | 13.9 |
| 2 | 101.4 | 13 | 4,443,926 | 7.8 |
| 3 | 112.2 | 14 | 5,352,423 | 8.0 |
| 4 | 64.4 | 10 | 6,718,284 | 6.4 |
| 5 | 75.4 | 13 | 5,308,870 | 5.8 |
| 6 | 77.2 | 7 | 3,655,516 | 11.0 |
| 7 | 90.7 | 10 | 7,148,014 | 9.1 |
| 8 | 75.5 | 8 | 9,048,054 | 9.4 |
| 9 | 78.3 | 14 | 5,215,817 | 5.6 |
| 10 | 90.0 | 13 | 4,964,849 | 6.9 |
| 11 | 72.3 | 14 | 3,965,902 | 5.2 |
| 12 | 75.3 | 17 | 4,058,084 | 4.4 |
| **Total** | 1,065.8 | 144 | 5,557,368 | 7.4 |

The overall genome coverage of the 12 tomato chromosomes was sufficient for detecting the majority of genomic regions associated with LB resistance in this mapping population. More than 70% of the genetic distances between markers were ≤10 cM (Fig. 5-3). However, several larger genetic gaps were discovered. The largest genetic distance

between markers was 63.0 cM, located near the top of chromosome 1 and caused by insufficient marker density in conjunction with high levels of recombination. Additionally, eight intervals of 30-40 cM were discovered on chromosomes 2, 3, 6, 7, 8, and 10, although distances of this size are unlikely to affect detection of LB resistance QTLs of moderate or large phenotypic effects (discussed below).


1
0.0 S006822
63.0 S027816
64.3 S036867
68.3 S037717
75.0 S037917
88.1 S038180 S038188
S038234
95.6 S038580
128.4 S039695
153.1 S040566
2
0.0 S041728
8.9 S044782 S045548
9.5 S048832
11.4 S053579
12.0 S054749
12.6 S054853
19.3 S055431
27.9 S055963
58.2 S057469
71.8 S057958 S057993
101.4 S058947
3
0.0 S059223
18.0 S059709
35.5 S061741 S062682
S063616 S064488
S067953
70.9 S073362
75.6 S073550
77.5 S073624
79.4 S073679
90.4 S074168
98.1 S074620
112.2 S075152
4
0.0 S076035
10.9 S080126 S081058
S083990
11.5 S086793
12.1 S087073
26.6 S088532
33.3 S088929
50.1 S089706
64.4 S090355
5
0.0 S091134
15.3 S091642
38.3 S092288
42.3 S092939
44.2 S093771 S097021
S097813
46.1 S102069
47.4 S105460
54.8 S106398
64.8 S106716
73.4 S106958
75.4 S106999
6
0.0 S115003
13.4 S118193
14.0 S118214
31.7 S118736
36.5 S118902
42.1 S119207
77.2 S119489

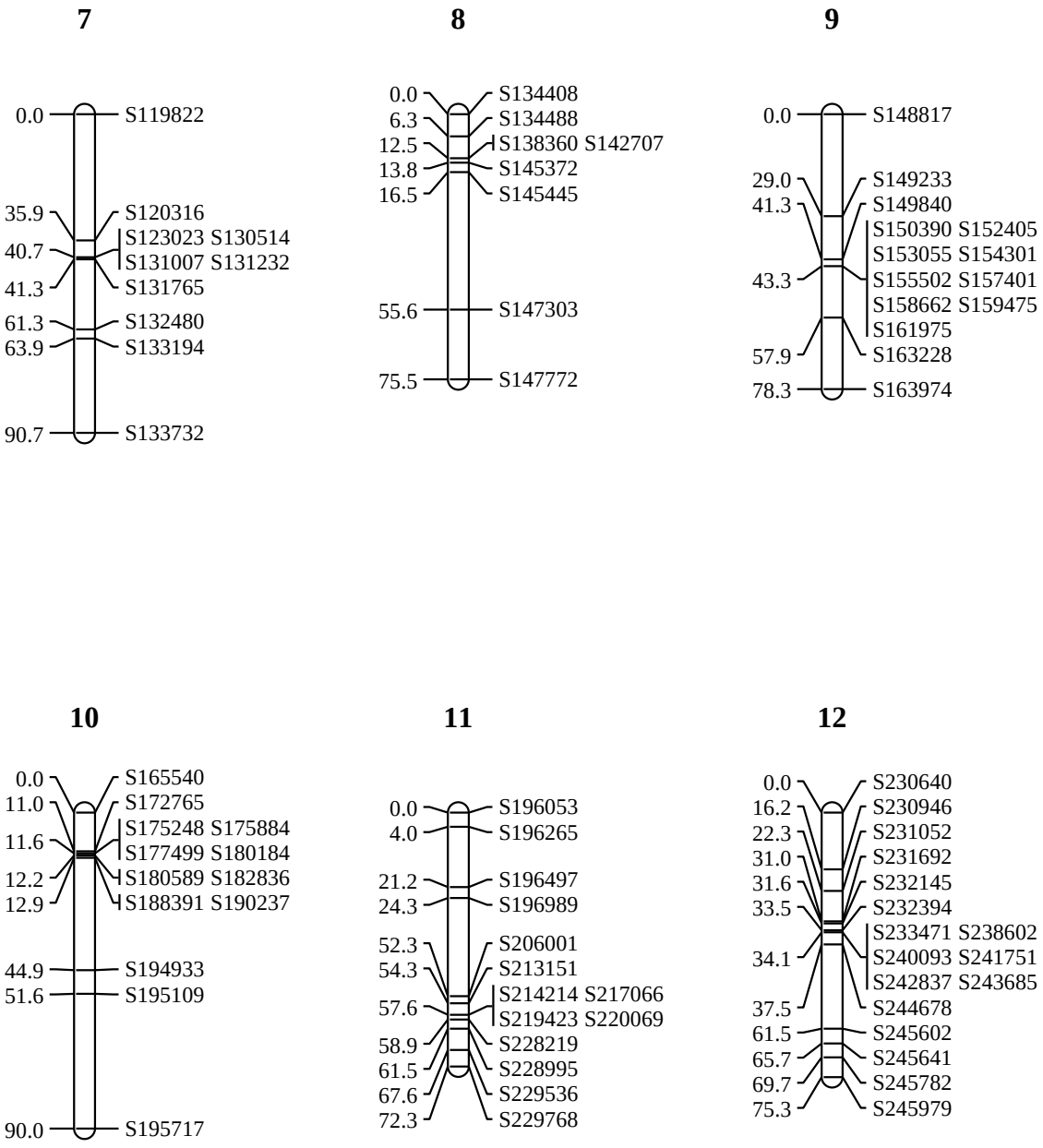



**Figure 5-3** $F_2$ genetic linkage groups derived from a cross between Fla. 8059 and PI 224710. A total of 80 $F_2$ individuals were genotyped with 144 SNP markers. Genetic mapping was performed using MapMaker 3.0 (LANDER *et al.* 1987).

**Marker segregation**

Chi-square ($X^2$) analyses were performed at each locus within each phenotypic class and several markers were identified that deviated from the expected 1:2:1 genotypic ratio. In the resistant class, genotype frequencies at 25 markers across 11 chromosomes exhibited skewed segregation (Table 5-4). In total, genotype frequencies for 14 markers located on chromosomes 1, 3, 5, 6, and 10 were skewed towards the PI 224710 genotype. The most extreme segregation was observed for markers on chromosomes 1 and 10, where individuals containing at least one PI 224710 allele accounted for between 91.7-100% of all resistant class genotypes.

Surprisingly, in the resistant class the genotype frequencies at 10 markers were skewed in favor of Fla. 8059 (Table 5-4). Genotype frequencies skewed in favor of Fla. 8059 in the resistant class were observed on chromosomes 2, 7, 8, 9, 10, 11 and 12. The highest levels of segregation occurred near the bottom of chromosome 7 and individuals containing at least one Fla. 8059 allele accounted for close to 90% of all resistant class genotypes. Additionally, in one instance the heterozygous genotype was heavily underrepresented, accounting for just 24.3% of the total genotypes at that locus (Table 5-4).

In the susceptible class, segregation at 21 markers significantly deviated from the expected Mendelian ratio (Table 5-4). At the majority of these loci, the frequency of the Fla. 8059 genotype was overrepresented, suggesting association of these markers with susceptibility. These regions were found on chromosomes 2, 9, 10, 11 and 12. The bottom of chromosome 10 was skewed most drastically in favor of Fla. 8059 and genotypes containing at least one Fla. 8059 allele accounted for between 81.6-100% of

the susceptible class individuals. The heterozygous genotype was overrepresented for eight markers on chromosome 12, accounting for as many as 75.0% of the susceptible class genotypes. Additionally, at each of these loci the homozygous PI 224710 genotypes were represented at less than half the frequency of those homozygous for Fla. 8059.

Only five markers segregated abnormally in both classes (Table 5-4). One marker at the top of chromosome 9 and two markers near the top of chromosome 11 were skewed in favor of the homozygous Fla.8059 genotype in both classes. No locus was skewed towards the PI 224710 genotype in both classes. However, two markers at the bottom of chromosome 10 in the resistant and susceptible classes were skewed in opposite directions in favor of the resistant and susceptible parental alleles respectively.

**Table 5-4** SNP locations and segregation in an $F_2$ mapping population (n = 599) derived from a cross between Fla. 8059 and PI 224710. Highly late blight resistant (n = 40) and susceptible (n = 40) $F_2$ individuals were genotyped with 144 markers. For each SNP marker, genotypes correspond to *pp* (homozygous PI 224710), *pq* (heterozygous), and *qq* (homozygous Fla. 8059). Chi-square ($X^2$) analyses were performed at each locus to determine if the genotype frequency fit the expected 1:2:1 Mendelian ratio for an $F_2$ population.

| Marker | Chromosome | Physical Locus (bp) | Genetic Locus (cM) | Resistant Class | | | | Susceptible class | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *pp* | *pq* | *qq* | $X^2$ (1:2:1) | *pp* | *pq* | *qq* | $X^2$ (1:2:1) |
| S006822 | 1 | 869,553 | 0.0 | 13 | 21 | 6 | 2.55 | 9 | 24 | 7 | 1.80 |
| S027816 | 1 | 51,992,555 | 63.0 | 12 | 20 | 6 | 2.00 | 7 | 21 | 11 | 1.05 |
| S036867 | 1 | 72,679,941 | 64.3 | 11 | 23 | 5 | 3.10 | 6 | 21 | 11 | 1.74 |
| S037717 | 1 | 76,215,183 | 68.3 | 15 | 18 | 6 | 4.38 | 6 | 21 | 13 | 2.55 |
| S037917 | 1 | 77,444,743 | 75.0 | 17 | 19 | 3 | 10.08* | 5 | 19 | 13 | 3.49 |
| S038180 | 1 | 79,762,738 | 88.1 | 15 | 20 | 5 | 5.00 | 10 | 17 | 13 | 1.35 |
| S038188 | 1 | 79,855,761 | 88.1 | 14 | 16 | 5 | 4.89 | 10 | 14 | 13 | 2.68 |
| S038234 | 1 | 80,145,027 | 88.1 | 15 | 20 | 5 | 5.00 | 10 | 17 | 13 | 1.35 |
| S038580 | 1 | 82,841,484 | 95.6 | 13 | 23 | 0 | 12.17* | 11 | 15 | 11 | 1.32 |
| S039695 | 1 | 89,731,813 | 128.4 | 7 | 24 | 4 | 5.34 | 7 | 21 | 7 | 1.40 |
| S040566 | 1 | 93,891,040 | 153.1 | 9 | 22 | 8 | 0.69 | 9 | 21 | 8 | 0.47 |
| S041728 | 2 | 537,665 | 0.0 | 8 | 17 | 13 | 1.74 | 3 | 16 | 19 | 14.42* |
| S044782 | 2 | 6,233,893 | 8.9 | 12 | 19 | 7 | 1.32 | 5 | 17 | 17 | 8.03* |
| S045548 | 2 | 7,421,251 | 8.9 | 9 | 19 | 8 | 0.17 | 5 | 15 | 16 | 7.72* |
| S048832 | 2 | 15,226,512 | 9.5 | 11 | 19 | 7 | 0.89 | 5 | 15 | 16 | 7.72* |
| S053579 | 2 | 25,809,518 | 11.4 | 13 | 15 | 6 | 3.35 | 6 | 13 | 17 | 9.5* |
| S054749 | 2 | 31,192,221 | 12.0 | 14 | 17 | 7 | 3.00 | 5 | 15 | 17 | 9.11* |
| S054853 | 2 | 32,920,145 | 12.6 | 16 | 17 | 7 | 4.95 | 6 | 17 | 16 | 5.77 |
| S055431 | 2 | 34,968,684 | 19.3 | 13 | 14 | 7 | 3.18 | 4 | 13 | 15 | 8.69* |
| S055963 | 2 | 37,415,171 | 27.9 | 12 | 17 | 6 | 2.09 | 4 | 24 | 12 | 4.80 |
| S057469 | 2 | 44,645,633 | 58.2 | 8 | 22 | 9 | 0.69 | 6 | 19 | 10 | 1.17 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S057958 | 2 | 47,655,240 | 71.8 | 5 | 21 | 14 | 4.15 | 9 | 21 | 10 | 0.15 |
| S057993 | 2 | 47,840,923 | 71.8 | 5 | 21 | 14 | 4.15 | 9 | 21 | 10 | 0.15 |
| S058947 | 2 | 53,864,779 | 101.4 | 4 | 14 | 19 | 14.35* | 9 | 14 | 14 | 3.54 |
| S059223 | 3 | 868,160 | 0.0 | 12 | 20 | 8 | 0.80 | 10 | 19 | 10 | 0.03 |
| S059709 | 3 | 3,702,920 | 18.0 | 15 | 17 | 8 | 3.35 | 11 | 16 | 11 | 0.95 |
| S061741 | 3 | 19,906,458 | 35.5 | 17 | 13 | 10 | 7.35* | 9 | 23 | 5 | 3.05 |
| S062682 | 3 | 25,024,646 | 35.5 | 17 | 13 | 10 | 7.35* | 9 | 22 | 7 | 1.16 |
| S063616 | 3 | 28,874,679 | 35.5 | 17 | 13 | 10 | 7.35* | 9 | 24 | 7 | 1.80 |
| S064488 | 3 | 31,405,225 | 35.5 | 16 | 13 | 10 | 6.18* | 8 | 23 | 7 | 1.74 |
| S067953 | 3 | 40,915,234 | 35.5 | 16 | 13 | 10 | 6.18* | 9 | 23 | 7 | 1.46 |
| S073362 | 3 | 61,353,196 | 70.9 | 11 | 18 | 10 | 0.28 | 10 | 20 | 9 | 0.08 |
| S073550 | 3 | 62,408,550 | 75.6 | 11 | 16 | 13 | 1.80 | 12 | 18 | 8 | 0.95 |
| S073624 | 3 | 62,874,374 | 77.5 | 10 | 13 | 15 | 5.11 | 12 | 15 | 10 | 1.54 |
| S073679 | 3 | 63,035,518 | 79.4 | 10 | 17 | 13 | 1.35 | 12 | 19 | 9 | 0.55 |
| S074168 | 3 | 65,027,754 | 90.4 | 11 | 18 | 10 | 0.28 | 12 | 18 | 9 | 0.69 |
| S074620 | 3 | 67,012,384 | 98.1 | 8 | 13 | 12 | 2.45 | 11 | 15 | 11 | 1.32 |
| S075152 | 3 | 70,449,660 | 112.2 | 7 | 19 | 11 | 0.89 | 9 | 21 | 9 | 0.23 |
| S076035 | 4 | 4,474,495 | 0.0 | 9 | 19 | 12 | 0.55 | 11 | 16 | 13 | 1.80 |
| S080126 | 4 | 18,403,976 | 10.9 | 12 | 22 | 5 | 3.15 | 11 | 20 | 8 | 0.49 |
| S081058 | 4 | 22,331,703 | 10.9 | 12 | 22 | 6 | 2.20 | 11 | 20 | 7 | 0.95 |
| S083990 | 4 | 35,883,127 | 10.9 | 12 | 22 | 6 | 2.20 | 11 | 20 | 9 | 0.20 |
| S086793 | 4 | 47,086,022 | 11.5 | 12 | 22 | 6 | 2.20 | 11 | 21 | 8 | 0.55 |
| S087073 | 4 | 48,328,855 | 12.1 | 10 | 23 | 6 | 2.08 | 11 | 21 | 8 | 0.55 |
| S088532 | 4 | 55,822,031 | 26.6 | 10 | 18 | 9 | 0.08 | 15 | 17 | 5 | 5.65 |
| S088929 | 4 | 59,296,245 | 33.3 | 8 | 21 | 10 | 0.44 | 15 | 17 | 5 | 5.65 |
| S089706 | 4 | 62,522,878 | 50.1 | 9 | 19 | 11 | 0.23 | 14 | 19 | 7 | 2.55 |
| S090355 | 4 | 64,939,052 | 64.4 | 8 | 21 | 11 | 0.55 | 9 | 22 | 7 | 1.16 |
| S091134 | 5 | 452,980 | 0.0 | 13 | 19 | 8 | 1.35 | 10 | 23 | 6 | 2.08 |
| S091642 | 5 | 3,179,941 | 15.3 | 14 | 22 | 4 | 5.40 | 11 | 23 | 4 | 4.26 |
| S092288 | 5 | 6,847,590 | 38.3 | 15 | 20 | 5 | 5.00 | 8 | 25 | 7 | 2.55 |
| S092939 | 5 | 10,198,437 | 42.3 | 16 | 17 | 6 | 5.77 | 10 | 23 | 7 | 1.35 |
| S093771 | 5 | 14,119,126 | 44.2 | 15 | 18 | 5 | 5.37 | 10 | 23 | 7 | 1.35 |
| S097021 | 5 | 29,610,944 | 44.2 | 15 | 18 | 6 | 4.38 | 10 | 23 | 7 | 1.35 |
| S097813 | 5 | 33,169,543 | 44.2 | 14 | 19 | 6 | 3.31 | 10 | 23 | 7 | 1.35 |
| S102069 | 5 | 47,056,460 | 46.1 | 14 | 20 | 5 | 4.18 | 9 | 25 | 5 | 3.92 |
| S105460 | 5 | 59,122,546 | 47.4 | 15 | 17 | 7 | 3.92 | 10 | 24 | 5 | 3.36 |
| S106398 | 5 | 61,498,446 | 54.8 | 17 | 13 | 6 | 9.5* | 11 | 20 | 5 | 2.44 |
| S106716 | 5 | 62,598,666 | 64.8 | 14 | 20 | 5 | 4.18 | 8 | 24 | 7 | 2.13 |
| S106958 | 5 | 63,969,295 | 73.4 | 16 | 19 | 5 | 6.15* | 11 | 22 | 7 | 1.20 |
| S106999 | 5 | 64,159,419 | 75.4 | 17 | 18 | 5 | 7.60* | 11 | 21 | 7 | 1.05 |
| S115003 | 6 | 25,472,372 | 0.0 | 9 | 21 | 10 | 0.15 | 10 | 20 | 7 | 0.73 |
| S118193 | 6 | 38,750,383 | 13.4 | 8 | 18 | 13 | 1.51 | 10 | 22 | 8 | 0.60 |
| S118214 | 6 | 38,826,424 | 14.0 | 8 | 19 | 13 | 1.35 | 9 | 23 | 8 | 0.95 |
| S118736 | 6 | 41,601,855 | 31.7 | 9 | 15 | 15 | 3.92 | 7 | 20 | 11 | 0.95 |
| S118902 | 6 | 42,813,562 | 36.5 | 9 | 16 | 15 | 3.40 | 7 | 20 | 12 | 1.31 |
| S119207 | 6 | 44,794,409 | 42.1 | 12 | 9 | 16 | 10.62* | 8 | 19 | 11 | 0.47 |
| S119489 | 6 | 47,405,467 | 77.2 | 12 | 24 | 3 | 6.23* | 12 | 24 | 4 | 4.80 |
| S119822 | 7 | 919,445 | 0.0 | 5 | 16 | 19 | 11.40* | 12 | 21 | 7 | 1.35 |
| S120316 | 7 | 4,133,718 | 35.9 | 8 | 20 | 12 | 0.80 | 13 | 19 | 6 | 2.58 |
| S123023 | 7 | 17,266,353 | 40.7 | 8 | 19 | 12 | 0.85 | 12 | 19 | 8 | 0.85 |
| S130514 | 7 | 48,242,934 | 40.7 | 7 | 20 | 12 | 1.31 | 12 | 20 | 8 | 0.80 |
| S131007 | 7 | 50,199,194 | 40.7 | 8 | 19 | 12 | 0.85 | 10 | 18 | 8 | 0.22 |
| S131232 | 7 | 51,141,453 | 40.7 | 8 | 20 | 12 | 0.80 | 12 | 19 | 8 | 0.85 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S131765 | 7 | 54,254,472 | 41.3 | 9 | 19 | 12 | 0.55 | 12 | 20 | 8 | 0.80 |
| S132480 | 7 | 59,742,785 | 61.3 | 4 | 22 | 13 | 4.79 | 11 | 21 | 7 | 1.05 |
| S133194 | 7 | 60,971,609 | 63.9 | 4 | 24 | 12 | 4.80 | 10 | 23 | 7 | 1.35 |
| S133732 | 7 | 65,251,571 | 90.7 | 4 | 18 | 16 | 7.68* | 10 | 19 | 9 | 0.05 |
| S134408 | 8 | 1,675,976 | 0.0 | 5 | 21 | 14 | 4.15 | 4 | 23 | 12 | 4.54 |
| S134488 | 8 | 2,284,340 | 6.3 | 6 | 19 | 15 | 4.15 | 6 | 18 | 14 | 3.47 |
| S138360 | 8 | 20,126,871 | 12.5 | 6 | 19 | 15 | 4.15 | 7 | 19 | 14 | 2.55 |
| S142707 | 8 | 38,970,761 | 12.5 | 6 | 18 | 15 | 4.38 | 6 | 19 | 14 | 3.31 |
| S145372 | 8 | 50,453,059 | 13.8 | 5 | 18 | 16 | 6.44* | 7 | 20 | 13 | 1.80 |
| S145445 | 8 | 50,817,661 | 16.5 | 8 | 13 | 16 | 6.73* | 9 | 18 | 13 | 1.20 |
| S147303 | 8 | 62,457,271 | 55.6 | 4 | 24 | 9 | 4.62 | 7 | 21 | 8 | 1.06 |
| S147772 | 8 | 65,012,353 | 75.5 | 9 | 22 | 9 | 0.40 | 9 | 21 | 9 | 0.23 |
| S148817 | 9 | 2,136,373 | 0.0 | 6 | 16 | 18 | 8.80* | 7 | 12 | 18 | 11.11* |
| S149233 | 9 | 3,645,130 | 29.0 | 7 | 27 | 5 | 5.97 | 7 | 22 | 8 | 1.38 |
| S149840 | 9 | 6,390,031 | 41.3 | 8 | 19 | 11 | 0.47 | 12 | 18 | 8 | 0.95 |
| S150390 | 9 | 9,102,976 | 43.3 | 10 | 19 | 11 | 0.15 | 12 | 20 | 8 | 0.80 |
| S152405 | 9 | 17,396,095 | 43.3 | 10 | 19 | 11 | 0.15 | 12 | 19 | 8 | 0.85 |
| S153055 | 9 | 22,152,953 | 43.3 | 10 | 18 | 11 | 0.28 | 12 | 20 | 8 | 0.80 |
| S154301 | 9 | 27,201,885 | 43.3 | 10 | 19 | 11 | 0.15 | 12 | 20 | 8 | 0.80 |
| S155502 | 9 | 31,264,469 | 43.3 | 10 | 19 | 11 | 0.15 | 12 | 20 | 8 | 0.80 |
| S157401 | 9 | 39,364,039 | 43.3 | 10 | 16 | 11 | 0.73 | 12 | 18 | 8 | 0.95 |
| S158662 | 9 | 43,130,366 | 43.3 | 10 | 19 | 10 | 0.03 | 12 | 20 | 8 | 0.80 |
| S159475 | 9 | 45,478,987 | 43.3 | 10 | 18 | 11 | 0.28 | 12 | 20 | 8 | 0.80 |
| S161975 | 9 | 57,608,575 | 43.3 | 10 | 19 | 11 | 0.15 | 12 | 20 | 8 | 0.80 |
| S163228 | 9 | 64,829,240 | 57.9 | 10 | 10 | 11 | 3.97 | 13 | 13 | 13 | 4.33 |
| S163974 | 9 | 69,941,999 | 78.3 | 11 | 19 | 9 | 0.23 | 9 | 9 | 17 | 11.91* |
| S165540 | 10 | 4,829,839 | 0.0 | 10 | 12 | 18 | 9.60* | 9 | 14 | 16 | 5.62 |
| S172765 | 10 | 17,120,812 | 11.0 | 11 | 19 | 9 | 0.23 | 9 | 19 | 11 | 0.23 |
| S175248 | 10 | 20,145,999 | 11.6 | 10 | 20 | 10 | 0.00 | 9 | 18 | 10 | 0.08 |
| S175884 | 10 | 21,614,104 | 11.6 | 10 | 19 | 8 | 0.24 | 9 | 20 | 11 | 0.20 |
| S177499 | 10 | 25,155,121 | 11.6 | 10 | 18 | 9 | 0.08 | 9 | 15 | 10 | 0.53 |
| S180184 | 10 | 29,684,639 | 11.6 | 10 | 20 | 8 | 0.32 | 8 | 20 | 11 | 0.49 |
| S180589 | 10 | 30,366,529 | 12.2 | 11 | 18 | 10 | 0.28 | 9 | 18 | 10 | 0.08 |
| S182836 | 10 | 33,678,153 | 12.2 | 11 | 19 | 9 | 0.23 | 8 | 20 | 11 | 0.49 |
| S188391 | 10 | 42,433,003 | 12.9 | 11 | 20 | 8 | 0.49 | 8 | 17 | 11 | 0.61 |
| S190237 | 10 | 46,487,297 | 12.9 | 11 | 20 | 8 | 0.49 | 8 | 18 | 11 | 0.51 |
| S194933 | 10 | 61,851,949 | 44.9 | 21 | 15 | 3 | 18.69* | 7 | 17 | 14 | 3.00 |
| S195109 | 10 | 63,024,556 | 51.6 | 25 | 12 | 1 | 35.47* | 4 | 19 | 15 | 6.37* |
| S195717 | 10 | 64,408,029 | 90.0 | 23 | 10 | 3 | 29.33* | 0 | 0 | 38 | 114* |
| S196053 | 11 | 576,180 | 0.0 | 10 | 16 | 14 | 2.40 | 6 | 19 | 15 | 4.15 |
| S196265 | 11 | 1,933,165 | 4.0 | 9 | 17 | 14 | 2.15 | 4 | 21 | 12 | 4.14 |
| S196497 | 11 | 3,614,670 | 21.2 | 10 | 10 | 18 | 11.89* | 4 | 16 | 16 | 8.44* |
| S196989 | 11 | 4,554,198 | 24.3 | 9 | 8 | 18 | 14.94* | 4 | 13 | 18 | 13.51* |
| S206001 | 11 | 20,484,488 | 52.3 | 9 | 19 | 10 | 0.05 | 7 | 18 | 13 | 2.00 |
| S213151 | 11 | 29,925,733 | 54.3 | 12 | 17 | 10 | 0.85 | 7 | 17 | 14 | 3.00 |
| S214214 | 11 | 32,112,889 | 57.6 | 14 | 15 | 10 | 2.90 | 6 | 18 | 13 | 2.68 |
| S217066 | 11 | 36,215,708 | 57.6 | 13 | 16 | 10 | 1.72 | 6 | 17 | 13 | 2.83 |
| S219423 | 11 | 39,178,924 | 57.6 | 14 | 16 | 10 | 2.40 | 6 | 18 | 13 | 2.68 |
| S220069 | 11 | 39,899,181 | 57.6 | 14 | 14 | 10 | 3.47 | 6 | 17 | 13 | 2.83 |
| S228219 | 11 | 48,922,550 | 58.9 | 12 | 16 | 10 | 1.16 | 7 | 18 | 14 | 2.74 |
| S228995 | 11 | 50,179,319 | 61.5 | 14 | 17 | 9 | 2.15 | 8 | 19 | 13 | 1.35 |
| S229536 | 11 | 51,516,133 | 67.6 | 14 | 17 | 9 | 2.15 | 9 | 16 | 15 | 3.40 |
| S229768 | 11 | 52,132,900 | 72.3 | 13 | 19 | 8 | 1.35 | 9 | 17 | 14 | 2.15 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S230640 | 12 | 209,922 | 0.0 | 8 | 14 | 17 | 7.26* | 6 | 16 | 13 | 3.06 |
| S230946 | 12 | 1,317,080 | 16.2 | 8 | 22 | 9 | 0.69 | 8 | 17 | 9 | 0.06 |
| S231052 | 12 | 1,811,385 | 22.3 | 8 | 22 | 7 | 1.38 | 7 | 19 | 5 | 1.84 |
| S231692 | 12 | 5,803,725 | 31.0 | 10 | 19 | 9 | 0.05 | 5 | 26 | 7 | 5.37 |
| S232145 | 12 | 8,291,475 | 31.6 | 11 | 21 | 8 | 0.55 | 5 | 24 | 8 | 3.76 |
| S232394 | 12 | 9,128,273 | 33.5 | 9 | 22 | 7 | 1.16 | 3 | 27 | 8 | 8.05* |
| S233471 | 12 | 15,230,552 | 34.1 | 10 | 21 | 7 | 0.89 | 1 | 27 | 8 | 11.72* |
| S238602 | 12 | 33,821,854 | 34.1 | 11 | 22 | 7 | 1.20 | 3 | 27 | 8 | 8.05* |
| S240093 | 12 | 40,454,923 | 34.1 | 11 | 22 | 7 | 1.20 | 4 | 26 | 8 | 6.00* |
| S241751 | 12 | 45,894,222 | 34.1 | 10 | 21 | 7 | 0.89 | 3 | 27 | 7 | 8.68* |
| S242837 | 12 | 51,478,012 | 34.1 | 11 | 22 | 5 | 2.84 | 3 | 25 | 8 | 6.83* |
| S243685 | 12 | 55,446,113 | 34.1 | 10 | 21 | 7 | 0.89 | 2 | 27 | 8 | 9.76* |
| S244678 | 12 | 58,138,136 | 37.5 | 12 | 20 | 7 | 1.31 | 1 | 27 | 8 | 11.72* |
| S245602 | 12 | 62,935,373 | 61.5 | 11 | 21 | 7 | 1.05 | 7 | 19 | 11 | 0.89 |
| S245641 | 12 | 63,371,069 | 65.7 | 12 | 20 | 8 | 0.80 | 7 | 21 | 11 | 1.05 |
| S245782 | 12 | 64,109,986 | 69.7 | 9 | 21 | 9 | 0.23 | 8 | 20 | 12 | 0.80 |
| S245979 | 12 | 65,139,260 | 75.3 | 9 | 20 | 11 | 0.20 | 8 | 18 | 12 | 0.95 |

*Significant at P ≤0.05

**Trait-based analysis (TBA)**

A trait-based analysis (TBA) approach was selected for mapping LB resistance. The PI 224710 marker allele frequency differences ($p_R$-$p_S$) between the resistant and susceptible classes were calculated and compared to the standard errors of the allele frequency differences ($\sigma_p$). Marker allele frequency differences $\geq 2\sigma_p$ indicated with at least 95% confidence a significant association between the marker locus and LB resistance. In this mapping population, 18 markers and four genomic regions were significantly associated with LB resistance conferred by PI 224710.

Several consecutive markers encompassing a 27.3 cM (6.6 Mbp) interval on chromosome 1 were significantly associated with resistance (Table 5-5). The allele frequency differences ranged from 0.16-0.29. A single marker at 75.0 cM was significantly associated with LB resistance at $>3\sigma_p$, providing >99% confidence this region is associated with LB resistance. In the resistant class, the PI 224710 allele ($p_R$) composed between 0.62 and 0.68 of the total alleles while in the susceptible class, PI 224710 alleles ($p_S$) accounted for 0.39-0.50 of the total alleles.

Near the top of chromosome 2, eight markers associated with LB resistance were identified between 8.9 cM and 27.9 cM, encompassing a 31.2 Mbp interval (Table 5-5). Four of these markers were associated with resistance at $>3\sigma_p$ between 11.4-19.3 cM, corresponding to a physical distance of 9.2 Mbp. In the resistant class, the allele frequency of PI 224710 ranged from 0.51-0.61, while in the susceptible class the Fla. 8059 allele frequency fell between 0.17 and 0.26.

Three markers on chromosome 10 were significantly associated with LB resistance and the marker allele frequency differences were the highest of any locus (Table 5-5). The region extended from 44.9-90.0 cM and the allele frequency difference increased from 0.32-0.78. All three markers were significant at $>3\sigma_p$. In the resistant class, the allele frequency of PI 224710 ranged from 0.73-0.82, while in the susceptible class the resistant allele frequency dropped from 0.41-0.0. While the genetic interval is more than 45 cM, the physical distance is only 2.6 Mbp demonstrating high recombination frequency. Since chromosome 10 extends an additional 1.1 Mbp past the final SNP marker mapped in this study, it is not possible to provide an exact genetic distance associated with this interval.

One marker on chromosome 12, S244678, was associated with LB resistance conferred by PI 224710. The marker was located at 37.5 cM and the allele frequency difference was 0.16. Allele frequency differences for several flanking markers were also slightly elevated, though not significantly. The distance between the two flanking markers was 27.4 cM and the physical distance associated with resistance was <7.5 Mbp. The PI 224710 allele frequency in the resistant class was slightly elevated (0.56) and in the susceptible class the PI 224710 allele frequency was 0.4. Although the allele

frequency differences were not particularly large, genotype segregation in the susceptible class was severely skewed. Less than 0.03 of the susceptible class individuals were homozygous for the PI 224710, suggesting this interval had a large effect on genotype segregation.

**Table 5-5** Loci significantly associated with late blight (LB) resistance. PI 224710 allele frequency differences were calculated between the resistant ($p_R$) and susceptible ($p_S$) classes and compared to the standard error of the marker allele frequency differences ($\sigma_p$). Allele frequency differences $\geq 2\sigma_p$ are considered associated with LB resistance at >95% confidence.

| Marker | Chromosome | Physical Locus (bp) | Genetic Locus (cM) | $p_R$ | $p_S$ | $p_R - p_S$ | $\sigma_p$ |
|---|---|---|---|---|---|---|---|
| S036867 | 1 | 72,679,941 | 64.3 | 0.58 | 0.43 | 0.14 | 0.08 |
| S037717 | 1 | 76,215,183 | 68.3 | 0.62 | 0.41 | 0.2* | 0.08 |
| S037917 | 1 | 77,444,743 | 75.0 | 0.68 | 0.39 | 0.29** | 0.08 |
| S038180 | 1 | 79,762,738 | 88.1 | 0.63 | 0.46 | 0.16* | 0.08 |
| S038188 | 1 | 79,855,761 | 88.1 | 0.63 | 0.46 | 0.17* | 0.08 |
| S038234 | 1 | 80,145,027 | 88.1 | 0.63 | 0.46 | 0.16* | 0.08 |
| S038580 | 1 | 82,841,484 | 95.6 | 0.68 | 0.50 | 0.18* | 0.08 |
| S039695 | 1 | 89,731,813 | 128.4 | 0.54 | 0.50 | 0.04 | 0.08 |
| S041728 | 2 | 537,665 | 0.0 | 0.43 | 0.29 | 0.14 | 0.08 |
| S044782 | 2 | 6,233,893 | 8.9 | 0.57 | 0.35 | 0.22* | 0.08 |
| S045548 | 2 | 7,421,251 | 8.9 | 0.51 | 0.35 | 0.17* | 0.08 |
| S048832 | 2 | 15,226,512 | 9.5 | 0.55 | 0.35 | 0.21* | 0.08 |
| S053579 | 2 | 25,809,518 | 11.4 | 0.60 | 0.35 | 0.26** | 0.08 |
| S054749 | 2 | 31,192,221 | 12.0 | 0.59 | 0.34 | 0.25** | 0.08 |
| S054853 | 2 | 32,920,145 | 12.6 | 0.61 | 0.37 | 0.24** | 0.08 |
| S055431 | 2 | 34,968,684 | 19.3 | 0.59 | 0.33 | 0.26** | 0.08 |
| S055963 | 2 | 37,415,171 | 27.9 | 0.59 | 0.40 | 0.19* | 0.08 |
| S057469 | 2 | 44,645,633 | 58.2 | 0.49 | 0.44 | 0.04 | 0.08 |
| S190237 | 10 | 46,487,297 | 12.9 | 0.54 | 0.46 | 0.08 | 0.08 |
| S194933 | 10 | 61,851,949 | 44.9 | 0.73 | 0.41 | 0.32** | 0.08 |
| S195109 | 10 | 63,024,556 | 51.6 | 0.82 | 0.36 | 0.46** | 0.07 |
| S195717 | 10 | 64,408,029 | 90.0 | 0.78 | 0.00 | 0.78** | 0.05 |
| S243685 | 12 | 55,446,113 | 34.1 | 0.54 | 0.42 | 0.12 | 0.08 |
| S244678 | 12 | 58,138,136 | 37.5 | 0.56 | 0.40 | 0.16* | 0.08 |
| S245602 | 12 | 62,935,373 | 61.5 | 0.55 | 0.45 | 0.11 | 0.08 |

*Marker allele frequency difference $\geq 2\sigma_p$
**Marker allele frequency difference $\geq 3\sigma_p$

Comparisons of the Fla. 8059 allele frequency differences to their standard errors identified four markers and three genomic regions that contributed to LB resistance (Table 5-6). A single locus associated with resistance was identified at 33.3 cM on chromosome 4. The allele frequency differences of the two flanking markers were also

elevated, indicating the resistance gene likely falls between 26.6 cM and 50.1 cM, which encompasses a 6.7 Mbp interval. The Fla. 8059 allele frequency (0.47) in the susceptible class was fairly close to the expected value based on Mendelian inheritance. However, in the resistant class the Fla. 8059 allele frequency was elevated at 0.64.

A genomic locus at the top of chromosome 7 exhibited the highest Fla. 8059 allele frequency difference between the resistant and susceptible class. The allele frequency difference (0.24) for the first marker on chromosome 7 was equal to $3\sigma_p$ (Table 5-6). This resistance QTL likely occurs within the first 4.1 Mbp, a genetic distance of approximately 35 cM. Two markers on the lower portion of chromosome 7 also had significantly elevated allele frequency differences from 61.3-90.7 cM, an approximately 5.5 Mbp interval. However, the frequency of Fla. 8059 alleles within this interval never exceeded 0.55 in the resistant class, suggesting its phenotypic effect is relatively small.

**Table 5-6** Fla. 8059 allele frequency in resistant ($q_R$) and susceptible ($q_S$) classes for genomic regions associated with late blight (LB) resistance. Allele frequency differences were calculated and compared to the standard error of the allele frequency differences ($\sigma_p$). Allele frequency differences $\geq 2\sigma_p$ are considered associated with LB resistance with at least 95% confidence.

| Marker | Chromosome | Physical Locus (bp) | Genetic Locus (cM) | $q_R$ | $q_S$ | $q_R - q_S$ | $\sigma_p$ |
|---|---|---|---|---|---|---|---|
| S088532 | 4 | 55,822,031 | 26.6 | 0.64 | 0.51 | 0.12 | 0.08 |
| S088929 | 4 | 59,296,245 | 33.3 | 0.64 | 0.47 | 0.16* | 0.08 |
| S089706 | 4 | 62,522,878 | 50.1 | 0.59 | 0.47 | 0.11 | 0.08 |
| S119822 | 7 | 919,445 | 0.0 | 0.56 | 0.33 | 0.24** | 0.08 |
| S120316 | 7 | 4,133,718 | 35.9 | 0.59 | 0.45 | 0.14 | 0.08 |
| S131765 | 7 | 54,254,472 | 41.3 | 0.55 | 0.46 | 0.09 | 0.08 |
| S132480 | 7 | 59,742,785 | 61.3 | 0.55 | 0.38 | 0.17* | 0.08 |
| S133194 | 7 | 60,971,609 | 63.9 | 0.54 | 0.40 | 0.14 | 0.08 |
| S133732 | 7 | 65,251,571 | 90.7 | 0.51 | 0.34 | 0.17* | 0.08 |

*Marker allele frequency difference $\geq 2\sigma_p$
**Marker allele frequency difference $\geq 3\sigma_p$

## Discussion

PI 224710 was highly resistant to LB in all experiments and not significantly different from resistant controls NC 870 (*Ph-3*) and NC 03220 (*Ph-2* + *Ph-3*) (Table 5-1).

Additionally, PI 224710 was significantly more resistant than NC 63EB (*Ph-2*). The high level of resistance in PI 224710 was confirmed in multiple greenhouse, field, and detached leaflet studies against several *P. infestans* isolates (FOOLAD *et al.* 2014a; FOOLAD *et al.* 2014b). Combined with the high heritability of LB resistance conferred by PI 224710 (discussed in Chapter 3), these results indicate that PI 224710 is likely a desirable source of LB resistance for tomato breeding. Furthermore, the $F_1$ progeny generation was also resistant to LB and not statistically different from PI 224710, suggesting the resistance could be potentially under dominant gene action. In contrast, Fla. 8059 was highly susceptible to LB and not statistically different from LB susceptible control lines NC 84173 or New Yorker.

The $F_2$ population ranged from 0-100% DS and was skewed slightly towards susceptibility (Fig. 5-1, Table 5-1). The $F_2$ population distribution is somewhat in discordance with the high level of resistance observed in the $F_1$ progeny and suggests the involvement of multiple resistance genes with additive effects and/or only partial dominance. This conclusion is also supported by mapping of multiple resistance QTLs, identified via TBA (discussed below). To fully characterize the nature of this resistance, development of near isogenic lines (NILs) is necessary to quantify the individual effects for each gene.

Previously, identifying sufficient numbers of polymorphic markers between *S. lycopersicum* and *S. pimpinellifolium* for mapping were often challenging due to relatively high genomic similarity. However, the availability of less expensive sequencing technologies and affordable SNP genotyping platforms has alleviated these issues. In this study, RRLs were constructed and sequenced for each parental line to

locate desirable SNP markers. This approach was highly successful, identifying more than 20,000 SNPs across all 12 chromosomes. Although not all SNPs were suitable for KASP marker development, 261 of the 373 SNPs were successfully converted. However, due to unpredictably high levels of heterozygosity in the PI 224710 parent utilized in development of the $F_2$ mapping population, only 144 were homozygous and polymorphic between Fla. 8059 and PI 224710. Consequently, the marker density of the genetic map was not as high as desired.

A genetic map was developed to verify the physical order of selected SNPs and examine recombination frequencies between markers (Fig. 5-3, Table 5-3). The genetic map corresponded perfectly with the physical order, which had previously been determined during genome assembly (Table 5-4). The genome length totaled 1,065.8 cM, consistent with previous estimates of tomato genome size based on maps developed between *S. lycopersicum* and *S. pimpinellifolium* (GRANDILLO AND TANKSLEY 1996; SHARMA *et al.* 2008; ASHRAFI *et al.* 2009; MERK *et al.* 2012; SIM *et al.* 2012). The genetic map contained eight genetic gaps between markers of 30-40 cM, as well as an interval of 63 cM between SNP markers on chromosome 1. However, genes of large phenotypic effects (>25%) would likely still be detected, and 30-40 cM intervals are unlikely to be sufficiently large enough to mask regions associated with even moderate (>9%) phenotypic effects (NAVABI *et al.* 2009). Furthermore, the average distance between markers across the entire genome was just 7.4 cM, which was more than adequate for detection of most resistance genes in the $F_2$ mapping population for which linkage disequilibrium (LD) was high.

To test for deviations of genotypic ratios within the resistant and susceptible classes, chi-square analyses were conducted at each marker locus. In total the genotypic frequencies at 41 markers differed significantly from the expected 1:2:1 ratio. Sixteen were associated with LB resistance QTLs or directly flanking regions associated with resistance (Tables 5-4, 5-5). Genotypic frequencies at only five markers were significantly skewed in both phenotypic classes. Two of these markers segregated in opposite directions between the resistant and susceptible classes and were significantly associated with LB resistance, while genotypes at the remaining three segregated in favor of Fla. 8059 in both classes, likely resulting from factors other than phenotypic selection. Skewed segregation has been reported extensively in tomato, especially in populations derived from interspecific crosses (GRANDILLO AND TANKSLEY 1996; CHEN AND FOOLAD 1999; LIPPMAN AND TANKSLEY 2001; ZHANG *et al.* 2003; SHARMA *et al.* 2008; ASHRAFI *et al.* 2009). Skewed segregation can be caused by phenotypic selection, self-incompatibility, unilateral incongruity, gametophytic selection, or viability selection (FOOLAD 1996; ASHRAFI *et al.* 2009). Since only the most resistant and susceptible phenotypes were genotyped, the majority of instances where abnormal segregation occurred are likely due to phenotypic selections. Nearly 40% of abnormally segregating SNPs occurred at or near regions associated with LB resistance, and for all but three of the remaining loci the abnormal segregation occurred in only one of the two phenotypic classes, suggesting phenotypic selection was the most likely cause. However, the majority of abnormal segregation was not sufficiently large to be detected by TBA.

Trait-based analysis identified four QTLs associated with LB resistance derived from PI 224710 and three genomic regions associated with resistance conferred by Fla.

8059. The strongest PI 224710 resistance QTLs appeared to be located on chromosomes 1, 2, and 10, each containing markers associated with LB resistance at >99% confidence. A Fla. 8059 allele frequency difference $>3\sigma_p$ was identified at a single locus near the top of chromosome 7. However, the other two genomic regions associated with LB resistance conferred by Fla. 8059 appeared to have had minor effects on the overall LB resistance.

The QTL identified on chromosome 1 is the second report of LB resistance located on this chromosome in *S. pimpinellifolium* (Table 5-5). Previously, the LB resistance gene *Ph-5* was mapped to the bottom of chromosome 1 in the *S. pimpinellifolium* accession PI 270443 (MERK *et al.* 2012). However, LB resistance in this region does not appear to correspond to the same interval identified by Merk *et al*. (2012). The resistance QTL mapped in PI 224710 corresponds to 76.2-82.8 Mbp, whereas the LB resistance QTL was not detected in PI 270443 until a marker located at 87.1 Mbp, suggesting these two QTLs do not correspond. This interval extends from 75.0-95.6 cM and consists of eight markers with allele frequency differences $\geq 2\sigma_p$ and includes one marker with an allele frequency difference $>3\sigma_p$. At one locus, all individuals within the resistant class contained at least one allele conferred by PI 224710, suggesting this QTL may have a large phenotypic effect. Additionally, there were a high number of heterozygous individuals within the resistant class suggesting the resistance is at least partially dominant. The resistance region contains 546 genes and four genes associated with plant defense, two of which encode CC-NBS-LRR class proteins (ITAG2.40, WWW.SOLGENOMICS.NET). The density of genes encoding CC-NBS-LRR proteins within the resistance interval is 0.30 genes/Mbp, compared to the chromosome 1 average of 0.04 genes/Mbp.

A resistance QTL was identified on chromosome 2, which consisted of eight markers and extended from 8.9-27.9 cM (Table 5-5). Four markers between 11.4-19.3 cM were associated with resistance at $>3\sigma_p$. The genomic region contains 926 genes, including 21 genes related to plant defense, three of which encode CC-NBS-LRR class proteins. No LB resistance QTLs of large phenotypic effect on LB resistance have previously been reported on chromosome 2. Brouwer *et al*. (2004) identified a resistance QTL, *lb2a*, in NC 84173 that explained as much as 24% of the resistance to *P. infestans* isolates from US-6 and US-11 clonal lineages. However, *lb2a* was mapped to a more proximal region than the resistance QTL identified in PI 224710. Additionally, NC 84173 was highly susceptible to the RS2009T1 isolate utilized in this study. A minor resistance QTL was mapped to chromosome 2 in L3708, the source of *Ph-3* (CHEN *et al.* 2014). The L3708 QTL is located near the bottom of the QTL reported in PI 224710. However, it is unknown if the resistance genes in PI 224710 and L3708 correspond. Late blight resistance on chromosome 2 has not previously been utilized in tomato breeding, suggesting this resistance locus on PI 224710 chromosome 2 could be valuable in tomato breeding.

The LB resistance QTL mapped to chromosome 10 appeared to account for the largest proportion of phenotypic variance based on allele frequency differences (Table 5-5). Three markers from 44.9-90.0 cM were significantly associated with resistance at $>3\sigma_p$ and allele frequency differences ranged from 0.32-0.78. Susceptible class genotypes for the final marker on chromosome 10 were all homozygous Fla. 8059. The delineated region contains 515 genes and the density of potential resistance genes is elevated at 2.34 genes/Mbp compared to the chromosome 10 average density of 0.73 genes/Mbp. The

region only contains a single CC-NBS-LRR class protein. The reported locus is consistent with previously reported LB resistance genes *Ph-2* and *Ph-5*, which were each mapped to chromosome 10 (GALLEGLY 1960; PEIRCE 1971; MOREAU *et al.* 1998; MERK *et al.* 2012). However, none of these genes have been fine mapped. Developing NILs and fine mapping the gene(s) responsible for LB resistance in this region are necessary to determine if resistance on chromosome 10 of PI 224710, *Ph-2*, and *Ph-5* are the same genes, different alleles of the same genes, or part of tightly linked resistance clusters, which have previously been reported for LB resistance genes in potato (PARK *et al.* 2005; PARK *et al.* 2009).

A single marker on chromosome 12, located at 37.5 cM was associated with LB resistance (Table 5-5). However, allele frequency differences >0.09 were identified for nine additional markers flanking the significant locus. Additionally, genotype segregation in the susceptible class was highly skewed for eight markers, with the homozygous PI 224710 genotype representing just 0.03-0.11 of the susceptible class across this interval. This region potentially accounts for a substantial portion of the LB resistance conferred by PI 224710 based on the highly distorted segregation in the susceptible class. However, the large number of heterozygous individuals in the susceptible class suggests that this gene is recessive in nature, reducing its utility in hybrid breeding. Additionally, fine mapping may be difficult due to its close proximity to the chromosome 12 centromere. The resistance QTL, *lb12b*, in the *S. habrochaites* accession LA2099 was previously identified in this interval. However, it is unknown if these two genes correspond. The tomato genome annotation (ITAG2.40) does not identify any genes related to plant defense within this region.

In total, four markers and three genomic regions were associated with increased LB resistance favoring the Fla. 8059 genotype (Table 5-6). However, it is suspected that these are largely due to stronger plant type and improved horticultural characteristics. One marker on chromosome 4 was associated with higher disease resistance, however this QTL does not contain any likely resistance genes. Fla. 8059 alleles were also favored in the resistant class for two genomic intervals on chromosome 7. The highest allele frequency difference was observed for a single marker at the top of chromosome 7 and was equal to $3\sigma_p$. This was largely accounted for by substantially lower frequencies of the Fla. 8059 allele in the susceptible class, while in the resistant class the allele frequency was only slightly elevated, suggesting the PI 224710 marker genotype at this locus contributed more to susceptibility than the Fla. 8059 marker genotype contributed to resistance. A similar effect was observed near the bottom of chromosome 7, with Fla. 8059 allele frequencies of <0.55 in the resistant class. Additionally, Fla. 8059 was highly susceptible in all experiments suggesting none of these QTLs were related to true host resistance (Table 5-1).

Unexpectedly, much of the PI 224710 parental genome utilized in developing the $F_2$ mapping population was heterozygous based on data collected during marker validation. Consequently, TBA may not have detected all resistance genes. For instance, if PI 224710 was heterozygous for both LB resistance and LB susceptibility at a particular locus, a SNP homozygous within PI 224710 may be unable to detect resistance as a result of overrepresentation of PI 224710 alleles in the susceptible class. However, since SNP density was generally high and only homozygous markers were utilized for

genetic map construction and TBA, most resistance QTLs were likely identified in this study.

The high level of LB resistance provided by PI 224710 and relatively few genomic intervals associated with resistance suggest that PI 224710 has good breeding potential for developing LB resistant tomato lines. One of the QTLs reported in this study was located on chromosome 1 and does not appear to correspond to previously reported LB resistance genes. Additionally, no chromosome 2 LB resistance QTLs have previously been released commercially, although it is unknown if the interval identified in this study corresponds with the minor QTL identified in L3708 (CHEN *et al.* 2014). The LB resistance on PI 224710 chromosome 10 appears to have the largest phenotypic effect on LB resistance. However, it is unknown if this corresponds to previously reported LB resistance genes *Ph-2* or *Ph-5* (GALLEGLY 1960; MOREAU *et al.* 1998; MERK *et al.* 2012; MERK AND FOOLAD 2012). The resistance reported in PI 224710 appears stronger than that conferred by *Ph-2* alone, although the % DS is usually somewhat higher than in PI 270443 (*Ph-5*) (FOOLAD *et al.* 2014b). However, the frequent identification of resistance on the bottom of chromosome 10 suggests this could be a resistance hotspot in *S. pimpinellifolium*. This region has also been identified in PI 163245 (Chapter 4), PI 270441, and PI 270442 (M.T. SULLENBERGER, unpublished data). Development of NILs is necessary to quantify the contribution of each resistance gene conferred by PI 224710 and identify the genomic regions most useful for breeding. Further delineation of the LB resistance genes via fine mapping is also necessary to identify tightly linked markers for marker assisted selection (MAS), which would enhance the speed and efficiency of incorporating and pyramiding these resistance genes

into tomato breeding lines. Development of NILs and introgression of PI 224710 LB resistance genes into tomato breeding material is currently being performed at The Pennsylvania State University.

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# Chapter 6 Conclusions and future prospects

Late blight (LB), caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most important diseases of tomato (*Solanum lycopersicum* L.) and potato (*Solanum tuberosum* L.) worldwide. Management of LB is notoriously difficult accounting for as much as 7% yield losses of tomato in the United States, and similar losses around the globe (NOWICKI *et al.* 2012). The impact of LB on potato is even more severe, accounting for approximately 16% yield losses annually worldwide (HAVERKORT *et al.* 2009). Currently, LB management depends primarily on frequent fungicide applications and good cultural practices. An alternative management strategy is the incorporation of new sources of genetic resistance to LB into tomato breeding lines. However, relatively few LB resistant cultivars are available commercially. Furthermore, qualitative disease resistance has proven unreliable due to rapid breakdown of *R* genes. Even *Ph-3*, currently considered the strongest tomato LB resistance gene, has been overcome by certain *P. infestans* isolates or under particularly high disease pressure (CHUNWONGSE *et al.* 2002; FOOLAD *et al.* 2008). In order to achieve durable and broad-spectrum LB resistance, pyramiding of qualitative resistance genes is desirable (MELCHINGER 1990; COLLARD AND MACKILL 2008). Consequently, identification of new sources of LB resistance has become a priority in tomato breeding.

## Disease response of parental accessions

Previously, the *S. pimpinellifolium* accessions PI 163245 and PI 224710 were identified as highly resistant to LB against multiple *P. infestans* isolates from clonal lineages US-13, US-14, and US-23 in field, greenhouse, and detached leaflet studies (FOOLAD *et al.* 2014a; FOOLAD *et al.* 2014b). Late blight resistance in these two

accessions was confirmed in several greenhouse experiments conducted within this study. PI 163245 was not statistically different from controls containing *Ph-2* or *Ph-3*, while PI 224710 outperformed controls containing only *Ph-2* and was not statistically different from controls containing *Ph-2* and *Ph-3* combined. While PI 224710 generally outperformed PI 163245 in this study, in two field evaluations PI 163245 was actually more resistant to LB than PI 224710, suggesting the phenotypic effects of these resistance genes are potentially related to plant maturity and/or disease pressure (FOOLAD *et al.* 2014b). This was especially apparent when comparing the area under disease progress curves (AUDPC), for which PI 163245 totaled 2.5 ± 2.5 % disease severity (DS) and PI 224710 reached 85.9 ± 76.2 % DS (FOOLAD *et al.* 2014b). However, overall both accessions were highly resistant. PI 163245 and PI 224710 were each more resistant to LB than L3708 (source of *Ph-3*) and controls containing *Ph-2* or *Ph-3* in field trials conducted in Pennsylvania and North Carolina (FOOLAD *et al.* 2014b; R.G. GARDNER, *pers. comm.*; M.R. FOOLAD *et al*., unpublished data). Consequently, incorporation of LB resistance genes from these two accessions into tomato breeding material is highly desirable.

Although both PI 163245 and PI 224710 displayed strong and similar levels of LB resistance, the mean % DS of the $F_1$ progeny generations suggested the genes responsible for resistance differ. While PI 163245 $F_1$ progeny average % DS (50.4% DS) was close to the mid-parental value (54.4 % DS), PI 224710 $F_1$ progeny averaged just 17.1% DS. The dominance exhibited by PI 224710 suggests this accession may be more valuable in hybrid breeding programs. However, further studies are necessary to

determine whether any of these genes are dominant as well as their overall phenotypic effects.

**Heritability of late blight resistance**

The heritability ($h^2$) of LB resistance conferred by PI 163245 and PI 224710 was estimated based on parent-offspring (P:O) correlation analyses and the realized heritability ($h^2_R$) was calculated. Estimates of $h^2$ based on $F_2$:$F_3$ P:O correlation analyses indicated LB resistance was highly heritable in multiple experiments for both PI 163245 and PI 224710, averaging 0.79 and 0.87 respectively. Since the PI 163245 $F_2$:$F_3$ experiments consisted of fewer $F_3$ families than desired, $F_3$:$F_4$ P:O correlation analysis was conducted to confirm the heritable nature of the resistance. Confirmation of P:O $h^2$ estimates were obtained via calculations of the realized heritability ($h^2_R$). Although measurements of $h^2_R$ were lower than the P:O estimates, in both cases $h^2_R$ averaged $\geq$0.59. The $h^2_R$ of PI 224710 ($h^2_R$ = 0.59) was slightly lower than PI 163245 ($h^2_R$ = 0.64). However, since for PI 163245 the $h^2_R$ was calculated based on the cumulative selection differential from $F_2$ to $F_4$ generations rather than $F_2$ to $F_3$, it is expected that $h^2_R$ would increase in successive generations for filial populations derived from PI 224710 (FALCONER AND MACKAY 1996). In fact, $h^2_R$ from $F_2$ to $F_3$ for PI 163245 averaged 0.56, which was slightly below the average $h^2_R$ for PI 224710. The moderately high levels of $h^2$ of LB resistance genes conferred by each of these accessions suggests that resistance is likely controlled qualitatively by a small number of genes in both PI 163245 and PI 224710, a conclusion which was supported by two methods of estimating the number of resistance loci (discussed in Chapters 2 and 3).

Estimates of $h^2$ in the $F_2$:$F_3$ experiments suggested the heritability of LB resistance in PI 224710 was higher than PI 163245. However, the low disease severity of PI 224710 $F_1$ progeny suggests dominance effects could have influenced the higher $h^2$ estimates. Since dominance effects in early filial populations can sometimes lead to inflated $h^2$ based on P:O regression/correlation, the $h^2$ of PI 224710 may have been overestimated (FOOLAD AND JONES 1992). However, the high P:O estimates of $h^2$ in conjunction with moderate $h^2_R$ suggest both accessions are suitable for breeding of LB resistance in tomato. Consequently breeding and mapping efforts were undertaken.

**Marker development and trait-based analysis**

Markers were developed by sequencing reduced representation libraries (RRLs) developed from Fla. 8059, PI 163245, and PI 224710. More than 20,000 SNPs were discovered between the two *S. pimpinellifolium* accessions and Fla. 8059. Approximately 70% of the 373 SNPs selected for marker development were successfully converted to Kompetitive Allele Specific PCR (KASP) markers. While marker development was only attempted for 373 SNP markers, the thousands of additional available SNPs will be useful for fine mapping the resistance genes reported in this study.

Genetic mapping of LB resistance was performed using a selective genotyping approach. $F_2$ populations derived from crosses between the two *S. pimpinellifolium* accessions and Fla. 8059 were developed. The most resistant ($n \geq 39$) and susceptible ($n \geq 35$) $F_2$ individuals were genotyped with SNP markers distributed throughout the genome and trait-based analysis (TBA) was performed. *S. pimpinellifolium* allele frequency differences between phenotypic classes were compared to the standard errors ($\sigma_p$) of the allele frequency differences. If the allele frequency differences were $>2\sigma_p$, the marker

was considered associated with LB resistance with at least 95% confidence. Four LB resistance QTLs were identified in each accession. Late blight resistance QTLs on chromosomes 2 and 10 co-localized in both accessions. Additionally, unique resistance QTLs were identified on PI 163245 chromosomes 3 and 11 and PI 224710 chromosomes 1 and 12.

**Chromosome 1**

A LB resistance QTL was mapped to a 27.3 cM region on PI 224710 chromosome 1, although this interval was not detected in the PI 163245 mapping population. At one locus, all resistant class individuals contained at least one PI 224710 allele. The resistance QTL appeared effective in both the homozygous and heterozygous states and heterozygous individuals accounted for 0.46-0.64 of resistant genotypes, outnumbering the homozygous PI 224710 individuals which ranged from 0.36-0.44. Although there were a substantial number of susceptible class individuals containing PI 224710 alleles, this was potentially influenced by heterozygosity of resistance in the PI 224710 parent since multiple $F_1$ individuals were used in generating sufficient $F_2$ seed. If PI 224710 was polymorphic for resistance and susceptibility, but the utilized markers were not, it would be possible for the PI 224710 marker genotype to be associated with both the resistant and susceptible classes.

Potential resistance genes were identified based on the tomato genome annotation, and the region contained two genes encoding CC-NBS-LRR class proteins (ITAG2.40, WWW.SOLGENOMICS.NET). Isolation of this resistance QTL in near isogenic lines (NILs) and further characterization is necessary for fine mapping and determining its utility in breeding. While six SNPs were mapped within this region, genetic gaps of 6.7 cM, 7.5

cM, and 13.1 cM were identified, necessitating the need to implement additional genetic markers. However, sequencing and comparison of the parental RRLs identified an additional 40 SNPs between PI 224710 and Fla. 8059 within this genomic interval, which were not utilized in this study and may prove useful for fine mapping.

Based exclusively on resistant class mapping data obtained in this study it appears the interval confers similar resistance as the QTL on chromosome 10 based on the extreme segregation of genotypes. Furthermore, since this resistance was not found in PI 163245 it potentially explains the higher level of resistance displayed by the PI 224710 $F_1$ progeny when compared to the PI 163245 $F_1$ progeny. While previously the LB resistance gene *Ph-5* was mapped to chromosome 1 in PI 270443 (MERK *et al.* 2012), the region identified in PI 224710 corresponds to a more proximal portion of the chromosome and does not appear to overlap with *Ph-5*, suggesting this is a novel resistance QTL.

**Chromosome 2**

A resistance QTL near the top of chromosome 2 was identified in both PI 163245 and PI 224710. The resistance QTLs mapped to similar intervals in both accessions, extending from 0-25.5 cM and 8.9-27.9 cM for PI 163245 and PI 224710 respectively. Previously, the minor resistance QTL, *qPh2.1* was mapped to the proximal portion of this region, though it is unknown if these resistance genes correspond to each other (CHEN *et al.* 2014). Although segregation of genotypes was not as extreme as found on chromosomes 1 and 10, the allele frequency differences exceeded $3\sigma_p$ at several loci. Additionally, the delineated region was fairly large (>19.0 cM), and detected in both mapping populations, suggesting these QTLs have at least moderate effects.

Genotype segregations were similar in both mapping populations. At markers with the highest allele frequency differences, the frequencies of homozygous Fla. 8059 individuals were only slightly diminished, accounting for 0.18 of the resistant class individuals. It is likely that the susceptible genotypes at this locus in the resistant class were masked by other resistance genes given the high level of resistance displayed by all $F_2$ parents and their $F_3$ progeny. However, there were several susceptible class genotypes which were homozygous for the PI 163245 and PI 224710 marker genotype. In both PI 163245 and PI 224710 mapping populations at least 0.06 of susceptible individuals were homozygous for the resistant genotype, suggesting this interval may not be sufficient to individually confer high levels of resistance.

In total, annotation of the tomato genome suggested more than 20 genes associated with plant defense occupied this region, including three that encode CC-NBS-LRR proteins. In PI 163245, the chromosome 2 and chromosome 10 resistance QTLs likely accounted for the majority of the observed LB resistance, since they were the only intervals detected over multiple markers and the highest allele frequency differences between classes were found within these regions. If the PI 163245 chromosome 10 resistance QTL corresponds to *Ph-2*, the chromosome 2 QTL warrants further study and consideration for incorporation into tomato breeding lines, since higher levels of resistance were observed in PI 163245 when compared to controls containing just *Ph-2* (FOOLAD *et al.* 2014b; R.G. GARDNER, *pers. commun*.). Although marker density was fairly high within this region, with genetic gaps between markers never exceeding 8.9 cM, the development of additional markers is necessary for saturating this genomic interval for fine mapping. More than 1,500 additional SNPs were discovered in this study

occupying this region between PI 163245 and Fla. 8059, and more than 400 SNPs were identified between PI 224710 and Fla. 8059, which is likely sufficient marker density for fine mapping purposes.

**Chromosome 3**

A LB resistance QTL was detected for a single marker (S073679) on PI 163245 chromosome 3 at 78.4 cM. However, in total seven marker allele frequency differences ≥0.12 were detected between 65.3 cM and 98.4 cM. The frequency of homozygous PI 163245 genotypes in the susceptible class was less than half the expected frequency, accounting for just 0.11 of the susceptible class individuals. However, annotation of the tomato genome found only one gene related to plant defense near this locus. While individually this resistance may not be effective, when combined with other resistance genes it could potentially increase the level or durability of LB resistance. However, it does not appear as promising as the resistance QTLs identified on PI 163245 chromosomes 2 or 10. Over 200 markers occupy the region surrounding this resistance locus if fine mapping is initiated, however mapping may be difficult if the phenotypic effect is as small as suspected.

**Chromosome 10**

Significant marker segregation was identified in both mapping populations on the distal portion of chromosome 10. The QTLs co-localized in both accessions, extending from 61.9 Mbp to near the end of the chromosome. In the susceptible classes, no *S. pimpinellifolium* alleles were found at the most distally located marker suggesting the resistance is somewhat effective in homozygous and heterozygous states. In the PI 163245 mapping population, as many as 0.92 of the resistant class genotypes were

homozygous for PI 163245 alleles. However, in the PI 224710 resistant class the number of homozygous PI 224710 individuals peaked at just 0.66, suggesting other QTLs (such as those identified on chromosomes 1 and 12) were contributing to the higher levels of resistance in the $F_1$ generation. This was one of the few loci in either mapping population for which homozygous genotypes outnumbered the heterozygous genotypes in the resistant class, suggesting these QTLs confer higher levels of LB resistance in the homozygous state. Nearly all resistant class individuals in both mapping populations contained at least one *S. pimpinellifolium* allele within this interval.

It is unknown if this resistance corresponds with previously identified LB resistance genes *Ph-2* and *Ph-5*, however all of these genes occupy the same distal portion of chromosome 10 (PEIRCE 1971; MOREAU *et al.* 1998; MERK *et al.* 2012). The bottom of chromosome 10 appears to be commonly associated with LB resistance in *S. pimpinellifolium* accessions and gaining a better understanding of the LB resistance associated with this region would be valuable for the scientific community. Including PI 163245 and PI 224710, resistance QTLs have been mapped to this region in at least six *S. pimpinellifolium* accessions (GALLEGLY 1960; PEIRCE 1971; MOREAU *et al.* 1998; MERK *et al.* 2012; M.T. SULLENBERGER, unpublished data). Fine mapping and cloning of these resistance genes are necessary for determining whether these are the same genes, alleles of the same genes, or part of a tightly linked *R* gene cluster such as those previously reported for LB resistance in potato (PARK *et al.* 2005; PARK *et al.* 2009). While *Ph-2* was mapped to an 8.4 cM interval on chromosome 10, further fine mapping has not been performed. More than 200 SNP markers were identified between PI 163245 and Fla.

8059 and >100 SNPs were found between PI 224710 and Fla. 8059 which may prove useful for fine mapping this important region.

**Chromosome 11**

The final late blight resistance QTL detected in PI 163245 was located on chromosome 11. This locus was not significantly associated with LB resistance in PI 224710. The homozygous PI 163245 genotype in the resistant class was 0.44, outnumbering the heterozygous genotype which was 0.38. This resistance QTL does not likely explain a large portion of the LB resistance in PI 163245, since the frequencies of the susceptible class individuals homozygous for the resistant genotype were >0.17, suggesting it is not an effective form of resistance individually and may not be as useful as the resistance genes detected on PI 163245 chromosomes 2 and 10. However, the majority of the resistant class contained at least one PI 163245 allele, suggesting it does contribute to the LB resistance conferred by PI 163245. The top of chromosome 11 also contains four genes that encode CC-NBS-LRR class proteins, suggesting at least one of these genes could explain the resistance detected in this region. Fine mapping of this region could be difficult due to the potentially small phenotypic effect size and the identification of only 25 additional SNPs occupying this region, so discovery of additional genetic markers could be necessary.

**Chromosome 12**

The last resistance QTL detected in PI 224710 was found on chromosome 12. Although LB resistance was only associated with a single SNP marker (S244678) at 37.5 cM, the allele frequency differences for nine additional markers flanking S244678 were slightly elevated. Although in the resistant class, segregation did not significantly differ

from the expected Mendelian ratio, the frequency of the homozygous susceptible genotype was slightly diminished at 0.18. In the susceptible class, segregation was significantly skewed in favor of the Fla. 8059 marker genotype. In fact, the frequency of the homozygous PI 224710 marker genotype in the susceptible class was just 0.03. In conjunction with the three additional LB resistance loci detected in PI 224710, the chromosome 12 resistance could be masked in the resistant class. Despite the smaller allele frequency differences between classes, the extreme segregation in the susceptible class would suggest the chromosome 12 resistance could have similar value as the LB resistance QTLs detected on chromosomes 1, 2, and 10. However, it appears the resistance QTL mapped to chromosome 12 is recessive in nature based on the high frequency of heterozygous genotypes in the susceptible class. Within the interval displaying skewed segregation in the susceptible class, 13 genes related to plant defense including two genes encoding CC-NBS-LRR proteins were identified. Fine mapping the chromosome 12 QTL may be difficult due to its close proximity to the centromere, which could inhibit rates of recombination. However, close to 2,000 SNPs were discovered within this region if fine mapping efforts are undertaken.

**Breeding and near-isogenic line development**

Near-isogenic line (NIL) development and backcross breeding efforts are currently in progress at The Pennsylvania State University. Several of the most resistant $F_2$ individuals in each mapping population were selected and self-pollinated to confirm resistance in $F_3$ progeny families. PI 224710 $F_3$ progeny with high levels of LB resistance were selected for backcross breeding and NIL development. In order to achieve higher levels of homozygosity, $F_3$ progeny developed from a cross between Fla. 8059 and PI

163245 were advanced another generation and $F_4$ progeny were selected for backcross (BC) breeding and NIL development.

Six highly resistant PI 163245 $F_4$ individuals derived from five $F_3$ families were backcrossed to Fla. 8059. Four of the $F_2$ parents were genotyped while conducting TBA and all except one were homozygous resistant or heterozygous for all the PI 163245 LB resistance QTLs reported in this study. Backcross progeny were selfed to develop $BC_1S_1$ families, but evaluation of these families for LB resistance and genotyping of resistant individuals using foreground and background markers are still needed.

Seven PI 224710 resistant $F_3$ progeny from seven different $F_3$ families were backcrossed to Fla. 8059. Five of the corresponding $F_2$ parents were genotyped while conducting TBA and four were homozygous or heterozygous for the resistant genotype for at least one marker within the four resistance QTLs identified in this study, while the remaining $F_2$ parent was only missing the resistant genotype for the chromosome 12 QTL. Two plants from each BC family were self-pollinated and the $BC_1S_1$ families were evaluated for LB resistance. Eight individuals including at least one from each $BC_1S_1$ family were selected based on their high levels of LB resistance and each $BC_1S_1$ individual was backcrossed to Fla. 8059. The $BC_2$ individuals were self-pollinated to develop $BC_2S_1$ families. However, evaluation of $BC_2S_1$ families for LB resistance and additional genotyping are still required.

Development of additional backcross generations and LB disease evaluations for subsequent generations are necessary to incorporate LB resistance genes into a uniform and stable genetic background. Genotyping of selfed backcross families with foreground and background markers will greatly facilitate and expedite the development of NILs.

Once each resistance QTL has been incorporated into a stable Fla. 8059 genetic background, fine mapping can be performed.

## Summary

Overall, TBA detected four resistance QTLs in each *S. pimpinellifolium* accession. Resistance QTLs on chromosomes 2 and 10 co-localized in both accessions, while the QTLs on chromosomes 3 and 11 were unique to PI 163245 and the QTLs on chromosomes 1 and 12 were detected only in PI 224710. Resistance QTLs on chromosomes 1, 2, and 10 each contained several marker allele frequency differences $>3\sigma_p$ suggesting these regions conferred the highest level of LB resistance and are likely the most suitable for tomato breeding purposes. Development of NILs to fine map and quantify the phenotypic effects of these resistance QTLs is currently underway.

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