INHERITANCE AND MAPPING OF RESISTANCE TO BACTERIAL SPOT RACE T4 (*Xanthomonas perforans*) IN TOMATO, AND ITS RELATIONSHIP TO RACE T3 HYPERSENSITIVITY, AND INHERITANCE OF RACE T3 HYPERSENSITIVITY FROM PI 126932

By

SAMUEL FORREST HUTTON

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To my sister, Catherine

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Soli Deo Gloria!

TABLE OF CONTENTS

page

ACKNOWLEDGEMENTS……..……..4

LIST OF TABLES……...6

LIST OF FIGURES……8

ABSTRACT……...10

CHAPTER

1 INTRODUCTION……..……12

2 INHERITANCE OF RESISTANCE TO XANTHOMONAS PERFORANS RACE T4 IN FLORIDA BREEDING LINES 8326, 8233 AND 8517……..…19

3 ANALYSIS OF MOLECULAR MARKERS FOR LINKAGE TO RESISTANCE LOCI IN FLORIDA BREEDING LINES 8233, 8517 AND 8326……46

4 GENETIC CONTROL OF RACE T3 HYPERSENSITIVITY FROM PI 126932 AND THE RELATIONSHIP BETWEEN RACE T3 HYPERSENSITIVITY AND RACE T4 RESISTANCE…….……87

APPENDIX

A PEDIGREES……..98

B ADDITIONAL MOLECULAR MARKER INFORMATION……..……..104

C DNA SOURCES FOR SELECTIVE GENOTYPING OF RESISTANT AND SUSCEPTIBLE SELECTIONS ……..……125

LIST OF REFERENCES……127

BIOGRAPHICAL SKETCH……..…..132

LIST OF TABLES

Table		Page
2-1	Bacterial spot race T4 disease severity for Florida 8326 (P_1), Florida 7946 (P_2), F_1, F_2, and backcross generations, and joint scaling tests for goodness of fit to an additive-dominance model	32
2-2	Estimates of additive, dominance, and interaction parameters for the Florida 8326 x Florida 7946 family	33
2-3	Bacterial spot race T4 disease severity for Florida 8233 (P_1), Florida 7776 (P_2), F_1, F_2, and backcross generations in spring 2007, and joint scaling test for goodness of fit to an additive-dominance model	34
2-4	Estimates of additive, dominance, and interaction parameters for the Florida 8233 x Florida 7776 family in spring 2007	35
2-5	Bacterial spot race T4 disease severity for Florida 8517 (P_1), Florida 7776 (P_2), F_1, F_2, and backcross generations in spring 2007, and joint scaling test for goodness of fit to an additive-dominance model	36
2-6	Estimates of additive, dominance, and interaction parameters for the Florida 8517 x Florida 7776 family in summer 2007	37
3-1	Markers polymorphic among genotypes resistant and susceptible to bacterial spot	67
3-2	Disease severity on resistant and susceptible selections from the Florida 7776 x Florida 8233 F_2 generation, and subsequent progeny in later seasons	80
3-3	Genotypic data on resistant and susceptible progeny selections (see Table 3-2) for markers polymorphic between Florida 8233 and Florida 7776	81
3-4	Disease severity on resistant and susceptible selections from the Florida 7776 x Florida 8517 F_2 generation, and subsequent progeny in later seasons	82
3-5	Genotypic data on resistant and susceptible progeny selections (see Table 3-4) for markers polymorphic between Florida 8517 and Florida 7776	83
3-6	Disease severity on resistant and susceptible selections from the Florida 7946 x Florida 8326 F_2 generation, and subsequent progeny in later seasons	85
3-7	Genotypic data on resistant and susceptible progeny selections (see Table 3-6) for markers polymorphic between Florida 8326 and Florida 7946	86

4-1 Hypersensitivity as measured by time to confluent necrosis in tomato plants infiltrated with *Xanthomonas perforans* race T3 in 2005……..95

4-2 Hypersensitivity to race T3 of *Xanthomonas perforans* on rooted tomato cuttings in fall 2005 and fall 2006…….96

4-3 Segregation of plants for bacterial spot race T3 hypersensitivity and race T4 field resistance in three F_2 families……97

B-1 Markers screened by a modified EcoTILLING approach to identify polymorphisms between PI 114490 and Florida 7776……105

B-2 Technical information for markers polymorphic among genotypes resistant and susceptible to bacterial spot……..110

B-3 Non-polymorphic markers……...123

C-1 DNA sources for selective genotyping of resistant and susceptible selections from Fla. 8233, Fla. 8517, and Fla. 8326 families……..126

LIST OF FIGURES

Figure — Page

2-1 Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8326, Fla. 7946, and generations derived from them at Citra, FL, during fall 2005. BC = backcross. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text)……..38

2-2 Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8326, Fla. 7946, and generations derived from them at Balm, FL, during spring 2006. BC = backcross. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text)……..39

2-3 Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8326, Fla. 7946, and generations derived from them at Citra, FL, during summer 2007. BC = backcross. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text)……..40

2-4 Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8233, Fla. 7776, and generations derived from them at Balm, FL, during spring 2007. BC = backcross. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text)……..41

2-5 Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8517, Fla. 7776 and generations derived from them at Citra, FL, during summer 2007. BC = backcross. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text)……..42

2-6 Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8233, Fla. 8326, and the F_2 generation derived from them at Citra, FL, during fall 2005 and spring 2006. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text)……..43

2-7 Bacterial spot race T4 disease severity frequency distribution for tomato parents Fla. 8517, Fla. 8233, and the F_2 generation derived from them at Citra, FL, during summer 2007. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text)……...44

2-8 Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8326, Fla. 8517, and the F_2 generation derived from them at Citra, FL, during summer 2007. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text)……...45

A-1. Pedigree of Fla. 8517. (Both Fla. 7655B and Fla. 7600 contain H7998 in their pedigrees.)……..……99

A-2. Putative pedigree of Fla. 8233. [However, PI 114490 is now thought to be incorrectly recorded in this pedigree. It appears that PI 128216 was actually crossed to Fla. 7655 (see Ch. 3).] (Fla. 7655 contains H7998 in its pedigree.)……...100

A-3. Pedigree of Fla. 8326. (Fla. 7708 contains H7998 in its pedigree.)……....101

A-4. Pedigree of Fla. 7776 …….……...…..102

A-5. Pedigree of Fla. 7946……..…….103

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INHERITANCE AND MAPPING OF RESISTANCE TO BACTERIAL SPOT RACE T4 (*Xanthomonas perforans*) IN TOMATO, AND ITS RELATIONSHIP TO RACE T3 HYPERSENSITIVITY, AND INHERITANCE OF RACE T3 HYPERSENSITIVITY FROM PI 126932

By

Samuel Forrest Hutton

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Resistance to bacterial spot of tomato (*Solanum lycopersicum)*, race T4 (*Xanthomonas perforans*) was characterized in three advanced breeding lines: Fla. 8326, Fla. 8233, and Fla. 8517; by generation means analysis (GMA). GMA of Fla. 8326 for two of three seasons (fall 2006 and summer 2007) indicated resistance is mostly dominant with significant additive and epistatic effects. GMA of Fla. 8233 in the spring of 2007 and of Fla. 8517 in the summer of 2007 also showed dominance to be the main effect in addition to additive and epistatic effects. Duplicate dominance or recessive suppressor type epistasis was indicated in each breeding line. Resistant (R) and susceptible (S) F_2 plants were selected from each of the three populations and the F_3 and F_4 progeny of these selections were evaluated to confirm resistance or susceptibility prior to including them for selective genotyping. Approximately 500 PCR-based markers, located primarily near areas of the genome where bacterial resistance genes have previously been identified, were screened to identify 269 polymorphic markers between *S. lycopersicum* and resistance sources. Polymorphic markers representing possible regions of introgression in each breeding line were analyzed for Transmission Disequilibrium (TD) across R and S selections

from each family to identify markers linked to resistance QTL. TD analysis indicated the following significant QTL: a PI 114490 resistance locus on chromosome 3 in Fla. 8517; a PI 128216 resistance locus on chromosome 9 in Fla. 8233 and Fla. 8517; a H7998/PI 128216 resistance locus on chromosome 11 in Fla. 8326, Fla. 8233, and Fla. 8517; and an OH9242 susceptibility locus on chromosome 12 in Fla. 8517. Non-significant but plausible QTL were indicated on chromosomes 1, 5 and 10. Race T3 hypersensitivity (HR) was controlled by a common locus in PI 126932 and PI 128216, which was different from the race T3 HR locus in H7981. Race T3 HR was inherited from PI 126932 as a single, dominant gene. Race T4 field resistance was not associated with race T3 HR in Fla. 8326, Fla. 8233 or Fla. 8517.

CHAPTER 1
INTRODUCTION

Fresh market tomato (*Solanum lycopersicum* L.) is the most valuable vegetable crop produced in Florida. The 2007-2008 tomato crop was harvested from 37,800 acres and was worth slightly over 464 million dollars (FASS, 2008). The number of harvested acres, although still very high, has declined over the past 15 years. Florida growers today face challenges from exotic diseases, encroaching urbanization, higher production costs, increased global competition, and the phase-out of methyl bromide. In response, growers are continually seeking ways to maximize efficiency by cutting costs and increasing yields. Yield reduction of tomatoes results from a number of factors, including adverse weather conditions, cultural problems, disease, insects, nematodes, and weeds. Disease is of particular significance in Florida because of the state's warm, humid climate. The most pervasive disease that faces Florida tomato production is bacterial spot.

Bacterial spot of tomato is caused by three species of Xanthomonas: *Xanthomonas euvesicatoria, X. vesicatoria,* and *X. perforans* (Jones, et al., 2000; Jones, et al., 2005); these species were formerly named *X. campestris* pv. *vesicatoria* and *X. vesicatoria* (Jones, et al., 2006). Optimum temperature for this bacterium's growth is around 27°C (Gardner and Kendrick, 1923), and secondary spread of the disease within plant beds and production fields occurs primarily by wind-driven rain, and surface-drainage of water that contains the bacterium (Sherf and Macnab, 1986). Thus, disease prevalence is greatest when temperatures are high and rainfall is frequent. Symptoms of this disease occur on leaves, stems, and fruit. Necrotic lesions generally appear as small well-defined spots. Leaves, however, may also take on a blighted or scorched appearance under conditions of heavy moisture, such as is experienced in Florida during the fall (Sherf and Macnab, 1986). Severe losses can occur due to leaf infections that

cause defoliation, which may result in both yield losses and reduced quality from cracking, sunscald, or black shoulder (Scott and Jones, 1986). Pohronezny and Volin (1983), documented that total marketable yield losses ranged from 17 to 52% under late and early bacterial spot infections, respectively. Bacterial spot especially reduced the number of USDA large size fruit (the fruit bringing the highest return to the producer).

Control of bacterial spot is based primarily on the use of bactericides, and has become more difficult over the years. Antibiotic sprays, particularly streptomycin, were once very efficacious in controlling the disease, but are no longer used due to the ability of the bacterium to develop resistance (Lai et al., 1977; Stall and Thayer, 1962). Copper formulations proved effective in reducing bacterial spot development (Stall, 1959). However, after years of application, many strains of the bacterium have developed copper resistance, and these resistant strains are now dominant in Florida (Marco and Stall, 1983). Copper applied in combination with mancozeb has been demonstrated to more effectively control bacterial spot than copper applied alone (Conover and Gerhold, 1981; Marco and Stall, 1983). Current control practices utilize this latter tank mix, but control is often poor during periods of high disease pressure (Jones and Jones, 1985; Jones et al. 1991a, b).

Because disease control is poor and there is concern about excessive use of pesticides, alternative methods for controlling bacterial spot have been researched. Compounds, such as Actigard, that induce systemic acquired resistance (SAR) in the plant have been reported as effective control alternatives (Louws et al., 2001), and Acigard is routinely used in north Florida against bacterial spot on field-grown fresh market tomato (Obradovic et al., 2005). The use of bacteriophages can also help control bacterial spot of tomato (Balogh et al., 2003; Flaherty et al.,

2000), and integrated use of SAR inducers and phages may complement each other as an alternative management strategy (Obradovic et al., 2005).

Much effort has been directed toward breeding for resistant varieties, but development has been difficult due to the limited availability of resistance sources, multigenic control of resistance, and the emergence of new races of the pathogen. Prior to 1989, all known strains from various areas of the world were of a single race (T1) of *X. euvesicatoria*. This race induced a hypersensitive response (HR) on the resistant genotype Hawaii 7998 (H7998), a small-fruited inbred bred for bacterial wilt resistance in Hawaii (Jones and Scott, 1986; Scott and Jones, 1986). Efforts were underway to incorporate this resistance into improved germplasm (Scott and Jones, 1989; Scott et al., 1991). Field resistance was largely additive and controlled by three to five effective factors (Scott and Jones, 1989). Whereas hypersensitive genes are generally thought to be single dominant genes, race T1 hypersensitivity from H7998 was initially determined to be associated with either two (Whalen et al., 1993) or three genes (Wang et al., 1994). Yu et al. (1995) subsequently identified three regions of the genome: *Rx-1* and *Rx-2*, located on the short and long arms of chromosome 1, respectively; and *Rx-3*, located on chromosome 5, confirming the multigenic control of race T1 hypersensitivity in H7998. Field resistance, however, was not explained by the hypersensitive response alone. Wang (1992) found correlation coefficients of only 0.39 to 0.41 between hypersensitivity and field resistance in two field F_2 populations. Likewise, Somodi et al. (1996) reported correlation coefficients of 0.31 to 0.52 between hypersensitivity and field resistance in two F_2 populations.

Race 2 (T2) (*X. vesicatoria*) first was identified from Brazil in 1989 (Wang et al., 1990), and later, it was found in several other locations around the world (Bouzar et al., 1994b; Stall et al., 1994). This new race did not induce a hypersensitive reaction on H7998 as did T1. In

addition, T2 strains were phenotypically different from T1 strains (Bouzar et al., 1994a, b; Jones et al., 1993) and were amylolytic and pectolytic, whereas T1 strains were not (Bouzar et al., 1994a; Stall et al., 1994). Strains of a third race (T3), *X. perforans*, which were classified originially as T2, were isolated in Florida beginning in 1991 and were described by Jones et al. (1995). The incorrect identification was due to the fact that these strains were also amylolytic and pectolytic, and they produced a compatible reaction on the tomato genotype H7998. However, unlike T1 and T2 strains, T3 strains induced a rapid hypersensitive response on the tomato genotype Hawaii 7981 (H7981) and *S. pimpinellifolium* accessions PI 126932 and PI 128216. In vitro studies found that T3 was antagonistic to T1 (El Morsy et al., 1994), and before T1-resistant cultivars could be developed in Florida, the T3 strain largely replaced T1 (Jones et al., 1998).

Scott et al. (1995) reported on the hypersensitivity and/or resistance of a number of lines to race T3 in 2 years of testing, including Hawaii 7981 (H7981), PI 128216, PI 126932, H7998, PI 114490, PI 155372, PI 340905-S and PI 126428. The former three lines all produced a hypersensitive reaction when infiltrated with *X. perforans* race T3, whereas the latter five did not. Of those lines producing race T3 hypersensitivity, H7981 showed the highest level of resistance, and selections of PI 126932 and PI 128216 had partial resistance. PI 114490, PI 155372, PI 340905-S and PI 126428 also displayed partial resistance, and H7998 exhibited a low level of tolerance. The hypersensitive response in H7981 was determined to be controlled by an incompletely dominant gene, *Xv3* (Scott et al., 1996), but field resistance was later determined to be quantitatively conferred by *Xv3* and other resistance genes (Scott et al., 2001). It is not known whether T3 hypersensitivity in PI 128216 and PI 126932 is conferred by *Xv3* or by a different

gene with similar function. One objective of this research is to determine the allelism of the T3 Hr genes in each of these sources.

A novel source of resistance to T3 was found in the wild species *S. pennellii* LA716 and described by Astua-Monge, et al. (2000a). This resistance, which causes a hypersensitive response with T3 strains, was determined to be different from *Xv3* in H7981, as previously described by Scott et al. (2001). LA716's resistance is conferred by *Xv4*, a resistance gene corresponding to the avirulence gene *avrXv4* in the pathogen (Astua-Monge, et al., 2000a).

Because of the potential for *X. vesicatoria* race T2 to emerge in Florida, resistance to this race, as well as to *X. euvesicatoria* race T1 and to *X. perforans race* T3 was desired. Scott et al. (1997) screened a number of tomato genotypes for resistance to race T2 and compared these data to published results for races T1 and T3. H7981 was highly resistant to race T3 but susceptible to races T1 and T2. PI 126932, PI 128216 and PI 126428 were also resistant to race T3 but susceptible to races T1 and T2. H7998, which was resistant to race T1, showed the same low level of tolerance to race T2 as it did to race T3. PI 155372 and PI 114490 were the only lines with desirable levels of resistance to all three races, with the latter having the highest and most consistent levels of resistance. Scott et al. (2003) reported on the inheritance of resistance from PI 114490 to race T2. This resistance was determined to be additive and controlled by two genes, where all four alleles were required for maximum resistance. A strong relationship between resistance genes for races T1 and T2 was observed, allowing for selection for resistance to either of these races to result in resistance to both races. Resistance to race T2 and race T3 segregated independently, indicating that race T3 resistance is not controlled by the same genes as race T2 resistance in PI 114490.

Recently, races T4 and T5 of *X. perforans* have emerged and appear to be associated with mutagenesis of tomato race 3 strains (Minsavage et al., 2003). The race T4 strains arose from mutations in the *avrXv3* gene, while the race T5 strains contain mutations in both the *avrXv3* and *avrXv4* genes. Race T4 strains overcame the hypersensitive resistance in H7981, PI 128216 and PI 126932 (Minsavage et al. 2003; Astua-Monge et al., 2000b), but have incompatible interactions when inoculated on LA716; whereas race T5 strains are compatible on genotypes containing *Xv3* and/or *Xv4*.

As Scott et al. (2003) pointed out, "a durable resistance in tomato that would be effective across races would be desirable." In particular, since races T3 and T4 are problems in Florida, resistance to both of these races is needed to prevent bacterial spot. Additionally, since race T1 might re-emerge if resistance to races T3, T4 and T5 was achieved, and race T2 could potentially become a problem in Florida, resistance to races T1 and T2 is also desireable. PI 114490 is resistant to race T4 (Scott, et al. 2006) as well as to the first three races of the pathogen, but little is known about the genetics resistance to race T4. Although race T4 overcame the T3 Hr resistance of PI 128216, this line has non-hypersensitive resistance to race T4 (Scott et al., 2006). Another goal of this research is to determine if the T3 hypersensitive resistance of this line plays a role in its T4 resistance. This study will also investigate T4 resistance from PI 114490, PI 128216 and PI 126932, and seek to determine whether any of these sources have common T4 resistance genes. One approach to answer this question will be to identify molecular markers linked to important resistant genes; these markers will also be very useful in efforts to pyramid resistance genes.

Three advanced breeding lines have been developed that carry resistance to race T4. Florida 8233 is a large-fruited fresh market tomato with PI 114490 and H7998 recorded in its

pedigree. It has a moderate to high level of resistance to race T4. Florida 8517, with PI 114490, PI 128216 and H7998 in its pedigree, is a plum tomato with moderate to high resistance. Florida 8326 has PI 126932 and H7998 in its pedigree. It is a large-fruited fresh market tomato with only a moderate level of resistance. To summarize, the objectives of this research were to 1) determine the inheritance of race T4 resistance from each of the three above resistant breeding lines, 2) identify molecular markers linked to resistance genes in each breeding line, 3) elucidate the inheritance of the race T3 hypersensitivity from PI 126932 and determine if it is conferred by the same locus as *Xv3* in H7981, and 4) investigate the relationship between race T3 HR and race T4 field resistance from Fla. 8233 and Fla. 8517.

CHAPTER 2
INHERITANCE OF RESISTANCE TO XANTHOMONAS PERFORANS RACE T4 IN FLORIDA BREEDING LINES 8326, 8233 AND 8517

Introduction

Bacterial spot of tomato (*Solanum lycopersicum* L.) is the most pervasive disease that faces Florida tomato production. Race T1, caused by *Xanthomonas euvesicatoria*, was the endemic race in Florida until 1991 when race T3 (*X. perforans*) emerged. The latter was antagonistic to (El Morsy et al., 1994) and largely replaced race T1 (Jones et al., 1998). Race T4 came about as a result of a mutation in the *X. perforans avrXv3* gene (Jones, unpublished), and has recently become prevalent (Jones, unpublished). Five races of the bacterial spot pathogen hae been identified without any selection pressure from monocultures of resistant cultivars. Despite this, host resistance still seems an attractive control strategy because bacterial spot is extremely difficult to control by chemical means, especially during hot, rainy weather common in Florida early in the fall production season.

Resistance to race T1 was identified in Hawaii 7998 (H7998) (Scott and Jones, 1986), and this resistance was based in part on hypersensitivity (Jones and Scott, 1986). Field resistance was not explained by hypersensitivity alone, but was largely additive and controlled by 3 to 5 effective factors (Scott and Jones, 1989). The race T1 hypersensitive response of H7998 was controlled by three regions of the genome (Wang et al., 1994; Yu et al., 1995), while field resistance was conferred by hypersensitivity and other genes (Wang, 1992; Somodi et al., 1996). Resistance to race T3 was identified in a number of sources, including hypersensitive resistance in H7981, PI 128216 and PI 126932 (Scott et al., 1995; Jones et al., 1995) and non-hypersensitive resistance in PI 114490 (Scott et al., 1995). Hawaii 7981 displayed the highest level of resistance to race T3, and field resistance was conferred by the incompletely dominant

resistance gene, *Xv3* (Scott et al., 1996), and other resistance genes (Scott et al., 2001). Race T4 overcame the *Xv3*-based hypersensitivity of H7981, PI 126932 and PI 128216 (Minsavage, et al., 2003; Astua-Monge et al., 2000b), but not the field resistance of PI 114490 or PI 128216 (Scott et al., 2006).

Ideally, resistance is needed that is effective across multiple races of bacterial spot. Particularly, since races T3 and T4 are problems in Florida, resistance to both of these races is needed to minimize damage associated with bacterial spot. However, little is known about the genetics of race T4 resistance. Three advanced breeding lines with resistance to bacterial spot races T3 and T4 have been developed (Scott et al., 2006). Florida 8326 is a large-fruited fresh market tomato with PI 126932 and H7998 in its pedigree; Florida 8233 is a large-fruited fresh market tomato with PI 128216 and H7998 in its pedigree; Florida 8517 is a plum tomato with PI 114490, PI 128216 and H7998 in its pedigree. Both Fla. 8233 and Fla. 8517 have moderate to high levels of resistance to race T4, while Fla. 8326 has only a moderate level of resistance to this race. The primary objective of this research was to determine the inheritance of resistance to race T4 in Florida breeding lines 8326, 8233 and 8517. A secondary objective was to evaluate the potential for combining resistance genes from each of these sources for a higher level of resistance to race T4.

Materials and Methods

Experimental Design, Inoculation and Disease Evaluation

Within each experiment, a randomized complete block design was used with four blocks, each with ten plants per plot for the parent, F_1 and reciprocal F_1 (RF_1) lines, and two plots of 25 plants for the F_2 generations. The RF_1 was included in each experiment to test for maternal inheritance of resistance. For all experiments, seed were sown in growth rooms in Black Beauty

spent coal (Reed Minerals Div., Highland, IN) and transplanted approximately 7 to 10 days later to Speedling® trays (3.8 cm^3 cell size) (Speedling, Sun City, FL) in the greenhouse, where seedlings were grown for four weeks. Plants were transplanted to field beds that were 20 cm high and 81 cm wide that had been fumigated with 67% methyl bromide : 33% chloropicrin at 197 kg ha^{-1} (175 lbs per acre) and covered with reflective plastic mulch. Plants were spaced 46 cm apart in rows, with 152 cm between rows, staked and tied, and irrigated by drip tape beneath the plastic mulch of each bed. A recommended fertilizer program was followed, and plants were sprayed with pesticides (excluding copper) as needed throughout the season (Olsen et al., 2007-2008).

Inoculum was produced by growing the bacterial strains on Difco nutrient agar (Becton Dickinson and Company, Sparks, Md.) for 24-36h at 28˚C. Bacterial cells were removed from the agar plates and suspended in 10 mM $MgSO_4 \cdot 7\ H_2O$, and the suspensions were standardized to A_{600} = 0.30 (a concentration of approximately 2 to 5 x 10^8 colony forming units (cfu)/mL). Inoculum was applied either at this concentration without surfactant, or was diluted to approximately 10^6 cfu/mL subsequent to standardization and applied along with Silwet L77 at 0.025% (v/v) as indicated below. Inoculum was applied by misting the foliage with a backpack sprayer. Plants were rated for disease severity in the field using the Horsfall and Barratt scale (1945), where 1 = 0%, 2 = 0%-3%, 3 = 3%-6%, 4 = 6%-12%, 5 = 12%-25%, 6 = 25%-50%, 7 = 50%-75%, 8 = 75%-87%, 9 = 87%-94%, 10 = 94%-97%, 11 = 97%-100%, and 12 = 100% diseased tissue. Data were subjected to generation means analysis (Mather and Jinks, 1982) using a spreadsheet program (Ng, 1990).

Plant Materials

Florida 8326

The race T4 susceptible inbred Fla. 7946 was crossed to Fla. 8326, and subsequently the F_1 was self-pollinated to produce F_2 seed and crossed to each parent to produce backcrosses. These generations were used for inheritance studies in fall 2005, spring 2006 and summer 2007. For the fall 2005 experiment, seed were sown on 29 July and transplanted to the field in Citra, FL on 9 September. Race T4 inoculum (at a concentration of approximately 2 to 5 x 10^8 cfu ml^{-1}) was applied to the plants early in the morning on 16 September, and each plant was rated for disease on 19 October. For the spring 2006 experiment, seed were sown on 17 February and transplanted to the field in Balm, FL on 29 March; race T3 inoculum (at a concentration of approximately 2 to 5 x10^8 cfu mL^{-1}) was applied to the plants early in the mornings on 5 May and again on 24 May because conditions were not favorable for disease during the first inoculation. However, subsequent race identification of plant lesions, and disease severity of control lines, each indicated that race T4 was responsible for field infection. Each plant was rated for disease the week of 26 June. For the summer 2007 experiment, seed were sown on 8 June, and race T4 inoculum (at a concentration of approximately 2 to 5 x 10^6 cfu mL^{-1}) was applied to plants on 20 July, prior to transplanting to the field in Citra, FL on 26 July. Each plant was rated for disease on 3 October.

Florida 8233

The race T4 susceptible inbred Fla. 7776 was crossed to Fla. 8233, and subsequently the F_1 was self-pollinated to produce F_2 seed and crossed to each parent to produce backcrosses. These generations were used for inheritance studies in fall 2006, spring 2007 and summer 2007. Extremely poor field conditions in fall 2006 and summer 2007 made disease severity ratings very difficult and resulted in unreliable data that will not be presented here. For the spring 2007 experiment, seed were sown on 1 February and transplanted to the field in Balm, FL on 13

March. Race T4 inoculum (at a concentration of approximately 2 to 5 x 10^8 cfu mL^{-1}) was applied early in the morning on 25 April, and each plant was rated for disease on 1 May.

Florida 8517

The race T4 susceptible inbred Fla. 7776 was crossed to Fla. 8517, and subsequently the F_1 was self-pollinated to produce F_2 seed and crossed to each parent to produce backcrosses. These generations were used for inheritance studies in fall 2006, spring 2007 and summer 2007. Extremely poor field conditions in fall 2006 and low disease levels in spring 2007 made disease severity ratings very difficult and resulted in unreliable data that will not be presented here. For the summer 2007 experiment, seed were sown on 8 June, and race T4 inoculum (at a concentration of approximately 2 to 5 x 10^6 cfu mL^{-1}) was applied to plants on 20 July, prior to transplanting to the field in Citra, FL on 26 July. Each plant was rated for disease on 3 October.

Combined resistance

Florida 8233 was crossed to Fla. 8326, and subsequently the F_1 was self-pollinated to produce F_2 seed. These generations were included in the Fla.8326 inheritance studies in Citra, FL in fall 2005 and in Balm, FL in spring 2006. Dates for seed sowing, transplanting, inoculation and disease evaluation were the same as those stated above for the Fla. 8326 fall 2005 and spring 2006 inheritance studies.

Florida 8326 was crossed to Fla. 8517, Fla. 8517 was crossed to Fla. 8233, and each F_1 was subsequently self-pollinated to produce F_2 seed. These generations were used for an inheritance study in summer 2007 in Citra, FL. Seed were sown on 8 June, and race T4 inoculum (at a concentration of approximately 2 to 5 x 10^6 cfu mL^{-1}) was applied to plants on 20 July, prior to transplanting to the field on 26 July. Each plant was rated for disease on 4 October.

Analysis of variance using the GLM procedure of the Statistical Analysis System (SAS Institute, Cary, N.C.) was used to test for differences between F_1 and RF_1 generations, and for differences between resistant parents.

Results

In two of the three seasons that Fla. 8326 was tested, the F_1 and RF_1 were not significantly different, indicative of nuclear inheritance; likewise, maternal inheritance was not indicated in Fla. 8233 or Fla. 8517 (data not shown). Thus within each family, the F_1 and RF_1 generations were combined for generation means analysis.

Florida 8326

Disease pressure was lower in fall 2005 than in either the spring 2006 or summer 2007 experiments as is evidenced by the higher mean disease severity of Fla. 7946 in each of the latter seasons (Table 2-1). In 2005, disease severities of the F_1 and F_2 were intermediate between the resistant and susceptible parents and skewed toward the susceptible parent (Figure 2-1). The mean of the BCP_1 was approximately equal to the midparent, and the BCP_2 mean was approximately equal to the susceptible parent (Tables 2-1 and 2-2). The fall 2005 data had an acceptable fit to an additive-dominance genetic model using the joint scaling test (Mather and Jinks, 1971) (Table 2-1), with only the additive effect having significance (Table 2-2). Broad sense heritability was estimated to be 0.66 by the method of Allard (1960); narrow sense heritability was estimated at 1.23 by the method of Warner (1952); and the method of Wright (1934) implicated one effective factor contributing to resistance (data not shown).

In spring 2006, disease severities of the F_1 and F_2 were intermediate between the resistant and susceptible parents and skewed toward the resistant parent (Figure 2-2). The BCP_1 was skewed toward the resistant parent as expected, but the BCP_2 segregated in a continuous pattern

(Figure 2-2) and had a lower mean than the midparent (Tables 2-1 and 2-2). In summer 2007, the F_1 and F_2 disease severities were both intermediate between the resistant and susceptible parents, but the F_2 mean disease severity was higher than would be expected under an additive-dominance model (Figure 2-3, Table 2-1). The BCP_1 mean was approximately equal to that of the F_1, while the BCP_2 had a lower mean than expected (Table 2-1). Neither the spring 2006 nor the summer 2007 data fit an additive-dominance model due primarily to deviations in the BCP_2 generation for both seasons and deviations in the F_2 generation in 2007 (Mather and Jinks, 1971) (Table 2-1).

Thus, an interaction analysis was performed that revealed significant homozygous x homozygous ([i]) interactions in summer 2007, and significant homozygous x heterozygous ([j]) and heterozygous x heterozygous ([l]) interactions in spring 2006 and summer 2007 (Table 2-2). Because the [h] and [l] parameters had opposite signs, duplicate dominance or recessive suppression type of epistasis was indicated (Mather and Jinks, 1982). Dominance and additivity were significant each season, and dominance had a greater effect. The presence of epistasis and dominance prevented the estimates of effective factors and heritabilities.

In fall 2005, 36 of 113 F_2 plants were as resistant as Fla. 8326 (disease severity ≤ 4), and 41 were as susceptible as Fla. 7946 (disease severity ≥ 6). In spring 2006, 54 of 146 F_2 plants were resistant, while only 10 were susceptible. In summer 2007, 21 of 152 F_2 plants were as resistant as Fla. 8326 (disease severity ≤ 5), and 41 were as susceptible as Fla. 7946 (disease severity ≥ 7). Averaged across the three seasons, approximately 27% of F_2 plants were resistant and 30% were susceptible.

Florida 8233

Disease pressure in spring 2007 was moderate. The disease severities of Fla. 8233 and Fla. 7776 were distinguishable from one another, but there was a low percentage of overlap between the two distributions (Figure 2-4). The F_1 was intermediate between resistant and susceptible parents and skewed toward resistance (Table 2-3, Figure 2-4). The BCP_1 had an excess of resistant plants, resulting in a lower mean disease severity than was expected. The BCP_2 was distributed between the two parents and was skewed slightly toward susceptibility. The F_2 showed continuous variation between the two parents. An additive-dominance model was not sufficient to explain the data, primarily due to deviations in the backcross generations (Table 2-3).

An interaction analysis revealed significant homozygous x homozygous ([i]) and heterozygous x heterozygous ([l]) interactions (Table 2-4).The opposite signs of the [h] and [l] parameters indicated duplicate dominance or recessive suppression type epistasis. Additive and dominance effects were also significant, and dominance had the greatest contribution to resistance. The presence of dominance and epistasis prevented the estimates of effective factors and heritabilities.

Of the 198 F_2 plants evaluated, 49 (25%) were as resistant as Fla. 8233 with disease severity ≤ 3. Forty-three (22%) were as susceptible as Fla. 7776 with disease severity ≥ 5.

Florida 8517

Under moderate to high disease pressure in summer 2007, parents separated with a small overlap. Mean disease severity of the F_1 and F_2 were intermediate between the resistant and susceptible parents, and each was skewed slightly toward susceptible (Table 2-5, Figure 2-5). The backcross to the resistant parent was more susceptible than expected, with a mean disease severity approximately equal to the F_1 and some very susceptible plants; and the BCP_2 was

slightly less susceptible than the susceptible parent. Deviations in the BCP_1 generation and, to a lesser extent, in the F_2 generation resulted in the inadequacy of an additive-dominance model to explain the data.

An interaction analysis identified significant, homozygous x homozygous ([i]), homozygous x heterozygous ([j]), and heterozygous x heterozygous ([l]) interactions, and duplicate dominance or recessive suppression type epistasis was again indicated (Table 2-6). Dominance and additive effects were significant, and dominance was the primary contributor to resistance.

One hundred sixty-three F_2 plants were evaluated in summer 2007. Of these, 37 (23%) were rated ≤ 4 and were as resistant as Fla. 8517, while 48 (30%) were rated ≥ 6 and were as susceptible as Fla. 7776.

Combined Resistance

In fall 2005, Fla. 8326 had a lower mean disease severity than Fla. 8233 ($P = 0.0024$), and in spring 2006, Fla. 8233 was more resistant than Fla. 8326 ($P < 0.0001$) (Figure 2-6). The F_2 generation had a mean disease severity intermediate between the two parents in both seasons. F_2 progeny disease severities were slightly skewed toward resistance in 2005 and 2006 and were distributed mainly within the range of the two parents. The F_2 distribution included one plant that was rated more resistant than either parent in 2005 and several F_2 plants in 2006 with slightly higher disease severity ratings than either parent.

In summer 2007, the parents Fla. 8517 and Fla. 8233 were approximately equal, and a fair number of individual plants were rated as susceptible (disease severity > 5) (Figure 2-7). The mean disease severity of the F_2 generation was similar to that of Fla. 8517. F_2 progeny did not

appear to segregate for individuals that were more susceptible than the parents, but the F_2 distribution included several plants with less disease than either parent.

Fla. 8517 was more resistant than Fla. 8326 in summer 2007 ($P = 0.0002$) (Figure 2-8), and both parents' distributions included individuals rated as susceptible (disease severity > 5). The F_2 progeny from the cross between these two lines had disease severities that were distributed primarily within the ranges of the two parents and slightly skewed toward Fla. 8326. Only 3 F_2 plants were identified with less disease than Fla. 8517, and none were more susceptible than either parent.

Discussion

The University of Florida's tomato breeding program has bred for bacterial spot resistance since the early 1980's. The main source of resistance to race T1 from H7998 was overcome in Florida by the emergence of race T3; H7981, PI 126932 and PI 128216 were each identified as resistant to the third race, and all three had race T3 hypersensitivity as well (Jones et al., 1995). Most of the breeding efforts for race T3 resistance focused on H7981 because this line had the highest level of resistance to race T3 (Scott et al., 1995), but PI 126932 and PI 128216 were also included in the breeding program to a lesser extent. Race T3 resistant breeding lines with these latter sources in their pedigrees include Fla. 8326, Fla. 8233 and one of the parents of Fla. 8517. Each of these three breeding lines also has non-hypersensitive race T4 resistance, indicating that they may have durable resistance. Results from inheritance studies presented in this research indicate that resistance in all three breeding lines is primarily dominant, but that epistasis is important and additive effects contribute significantly.

Although both Fla. 7776 and Fla. 7946 are susceptible to bacterial spot, Fla. 7946 is much more susceptible than Fla. 7776. Both were used as susceptible parents to study

inheritance. Resistant and susceptible individuals were easily distinguished in the Fla. 8326 x Fla. 7946 cross, even in fall 2005 when disease pressure was low. This is in contrast to crosses involving Fla. 7776, where resistant and susceptible parents still separated, but with a small percentage of overlap. This emphasizes the importance of choosing parents with adequate separation, especially when working with a quantitative trait.

In fall 2005, under low bacterial spot disease pressure, Florida 8326 inheritance data fit an additive-dominance model. However, an erroneous narrow-sense heritability was calculated. Two of the assumptions for Warner's (1952) method of calculating this heritability are the absence of dominance and the absence of epistasis. The presence of these effects in Fla. 8326, although undetected in fall 2005, is possibly the reason for the overestimation of narrow-sense heritability, since both dominance and epistasis were significant in the Fla. 8326 spring 2006 and summer 2007 inheritance studies. Additionally, one effective factor was identified in fall 2005. It may be that under low disease pressure, a single gene in Fla. 8326 can provide acceptable resistance, but that under higher pressure, a second, interacting gene is needed. This concept will be discussed further in Chapter 3.

Combining resistance from various sources is an approach for developing durable resistance, as well as for increasing the level of achievable resistance. Each of the resistant breeding lines used in this study was crossed with one another to determine the potential for pyramiding their resistance genes and to estimate whether these lines had QTL in common. In general, susceptible plants did not segregate from the F_2 generations of the two crosses involving Fla. 8326; nor were segregates identified that were more resistant than either parent. This suggests that resistance in Fla. 8326 is based on QTL in common with Fla. 8233 and Fla. 8517, and that additional resistance genes contribute to the higher level of resistance in the latter two

lines, thus limiting the potential for pyramiding genes from Fla. 8326. By this hypothesis, it follows that susceptible segregates were not identified in the F_2 between Fla. 8517 and Fla. 8233. However, the identification of a 6 F_2 plants from this cross with lower disease severity ratings than either parent supports the possibility that these two lines may not have all QTL in common. This is not certain, since the identification of these individuals may simply be the result of the large population size of the F_2 relative to the parents. These plants were selected and are being tested to determine if they are, in fact, more resistant than either parent.

The multigenic control of the partial resistance to race T4 from Fla. 8326, Fla. 8233 and Fla. 8517 presents significant difficulties for developing resistant cultivars. Although the F_1 was an improvement over the susceptible parent in each inheritance study, this would probably not be a commercially acceptable level of resistance with the partially resistant parents used. Thus, it will be necessary to incorporate this resistance into both parents of a hybrid. Furthermore, incorporation of this resistance into lines that are not highly susceptible (such as Fla. 7776) will be particularly difficult, since progeny with moderate and higher levels of resistance are not easily distinguishable. This, combined with the complicating effects of dominance and epistasis, necessitates that selections be evaluated in subsequent trials to obtain plants homozygous for resistant alleles.

The potential for pyramiding resistance QTL from Fla. 8326, Fla. 8233 and Fla. 8517 for durable resistance depends, in part, on the source of each QTL. Evidence from complementation tests suggests that Fla. 8326 has limited usefulness in pyramiding, but that it may be possible to pyramid resistance genes from Fla. 8233 and Fla. 8517. Each of these resistant breeding lines has two or more potential donors of resistance in its pedigree, but it is uncertain which donors are contributing to resistance. Chapter 3 discusses the identification of molecular markers linked to

resistance QTL in each line and addresses the source of each QTL. The use of molecular markers linked to resistance QTL will also be extremely useful in applied breeding work to overcome the aforementioned difficulties in obtaining plants homozygous for resistance alleles.

Table 2-1. Bacterial spot race T4 disease severity for Florida 8326 (P_1), Florida 7946 (P_2), F_1, F_2, and backcross generations, and joint scaling test for goodness of fit to an additive-dominance model.

			Mean disease severity[z]			
Season	Generation	Plant no.	Observed	Expected	Variance	Goodness of Fit
Fall 2005	P_1	30	3.65	3.66	0.49393	0.019
	BCP_1	35	4.49	4.41	0.57504	0.658
	F_1	65	5.16	5.16	0.69094	0.001
	F_2	113	4.88	4.97	0.99895	0.972
	BCP_2	48	5.55	5.54	0.56990	0.021
	P_2	36	5.93	5.91	0.53656	0.028
						$X^2 = 1.70$
						$P = 0.64$
Spring 2006	P_1	35	3.55	3.54	0.16471	0.007
	BCP_1	76	3.93	3.87	0.66895	0.633
	F_1	54	4.48	4.19	0.64450	9.939
	F_2	146	4.72	4.83	0.91003	2.846
	BCP_2	77	5.29	5.80	0.89560	27.515
	P_2	37	7.69	7.40	0.33821	9.778
						$X^2 = 50.72$
						$P < 0.0001$
Summer 2007	P_1	23	4.50	4.76	0.18182	8.357
	BCP_1	54	5.24	5.03	0.57303	4.127
	F_1	66	5.20	5.29	0.29213	1.812
	F_2	157	6.33	5.80	0.95317	47.445
	BCP_2	74	6.20	6.56	0.38986	24.335
	P_2	25	7.92	7.83	0.18083	1.035
						$X^2 = 87.11$
						$P < 0.0001$

[z] Rated on the Horsfall-Barratt (1945) scale. Higher number indicates more disease, see text.

Table 2-2. Estimates of additive, dominance and interaction parameters for the Florida 8326 x Florida 7946 family.

Season	Parameter[z]	Estimate (±SE)	*t* test
Fall 2005	m	4.230 ± 0.471	8.98 ***
	[d]	-1.140 ± 0.064	-17.68 *
	[h]	1.670 ± 1.137	1.47 NS
	[i]	0.560 ± 0.467	1.20 NS
	[j]	0.160 ± 0.303	0.53 NS
	[l]	-0.740 ± 0.700	-1.06 NS
Spring 2006	m	6.028 ± 0.432	13.95 ***
	[d]	-2.070 ± 0.060	-34.69 **
	[h]	-3.697 ± 1.091	-3.39 **
	[i]	-0.408 ± 0.428	-0.95 NS
	[j]	1.420 ± 0.312	4.56 **
	[l]	2.146 ± 0.703	3.05 **
Summer 2007	m	8.662 ± 0.408	21.24 ***
	[d]	-1.710 ± 0.006	-27.21 *
	[h]	-5.854 ± 1.006	-5.82 **
	[i]	-2.452 ± 0.403	-6.09 **
	[j]	1.495 ± 0.284	5.27 **
	[l]	2.396 ± 0.624	3.84 **

[z] Definitions: m = midpoint (between AA and aa), [d] = difference of AA and aa from midparent, [h] = difference of Aa from midparent value, [i] = homozygote x homozygote interaction, [j] homozygote x heterozygoe interaction, and [l] heterozygote x heterozygote interaction.

NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2-3. Bacterial spot race T4 disease severity for Florida 8233 (P_1), Florida 7776 (P_2), F_1, F_2, and backcross generations in spring 2007, and joint scaling test for goodness of fit to an additive-dominance model.

		Mean disease severity[z]			
Generation	Plant no.	Observed	Expected	Variance	Goodness of Fit
P_1	40	3.35	3.19	0.19939	4.679
BCP_1	100	3.04	3.41	0.58929	23.465
F_1	80	3.78	3.64	0.24156	7.245
F_2	200	3.99	3.97	0.81716	0.086
BCP_2	100	4.23	4.53	0.73473	11.828
P_2	40	5.60	5.42	0.38394	3.342
					$X^2 = 50.65$
					$P < 0.0001$

[z] Rated on the Horsfall-Barratt (1945) scale. Higher number indicates more disease, see text.

Table 2-4. Estimates of additive, dominance and interaction parameters for the Florida 8233 x Florida 7776 family in spring 2007.

Parameter[z]	Estimate (±SE)	*t* test
m	5.901 ± 0.352	16.75 ***
[d]	-1.128 ± 0.061	-18.45 *
[h]	-5.529 ± 0.890	-6.21 ***
[i]	-1.427 ± 0.347	-4.11 *
[j]	-0.116 ± 0.264	-0.44 NS
[l]	3.413 ± 0.558	6.11 ***

[z] Definitions: m = midpoint (between AA and aa), [d] = difference of AA and aa from midparent, [h] = difference of Aa from midparent value, [i] = homozygote x homozygote interaction, [j] homozygote x heterozygoe interaction, and [l] heterozygote x heterozygote interaction.

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2-5. Bacterial spot race T4 disease severity for Florida 8517 (P_1), Florida 7776 (P_2), F_1, F_2, and backcross generations in summer 2007, and joint scaling test for goodness of fit to an additive-dominance model.

		Mean disease severity[z]			
Generation	Plant no.	Observed	Expected	Variance	Goodness of Fit
P_1	34	3.99	4.07	0.28008	0.793
BCP_1	76	5.09	4.64	0.72259	20.785
F_1	78	5.17	5.21	0.27160	0.358
F_2	163	4.99	5.20	0.63417	11.508
BCP_2	91	5.92	5.77	0.49957	4.328
P_2	34	6.32	6.33	0.33155	0.002
					$X^2 = 37.77$
					$P < 0.0001$

[z] Rated on the Horsfall-Barratt (1945) scale. Higher number indicates more disease, see text.

Table 2-6. Estimates of additive, dominance and interaction parameters for the Florida 8517 x Florida 7776 family in summer 2007.

Parameter[z]	Estimate (±SE)	*t* test
m	3.100 ± 0.358	8.67 ***
[d]	-1.169 ± 0.068	-17.17 **
[h]	5.489 ± 0.918	5.98 ***
[i]	2.054 ± 0.351	5.85 **
[j]	0.663 ± 0.282	2.35 *
[l]	-3.416 ± 0.582	-5.87 **

[z] Definitions: m = midpoint (between AA and aa), [d] = difference of AA and aa from midparent, [h] = difference of Aa from midparent value, [i] = homozygote x homozygote interaction, [j] homozygote x heterozygoe interaction, and [l] heterozygote x heterozygote interaction.

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

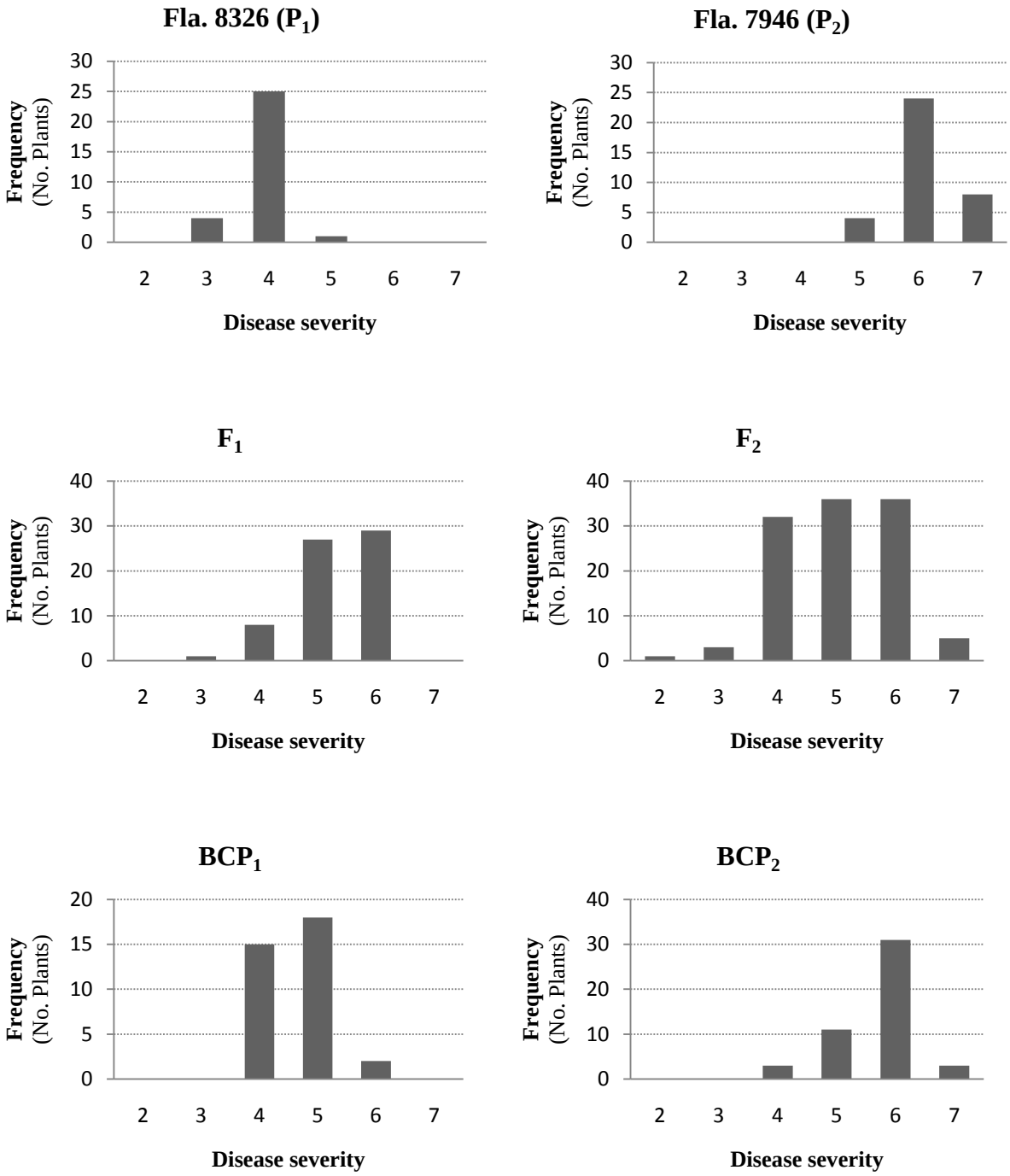


Figure 2-1. Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8326, Fla. 7946, and generations derived from them at Citra, FL, during fall 2005. BC = backcross. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text).

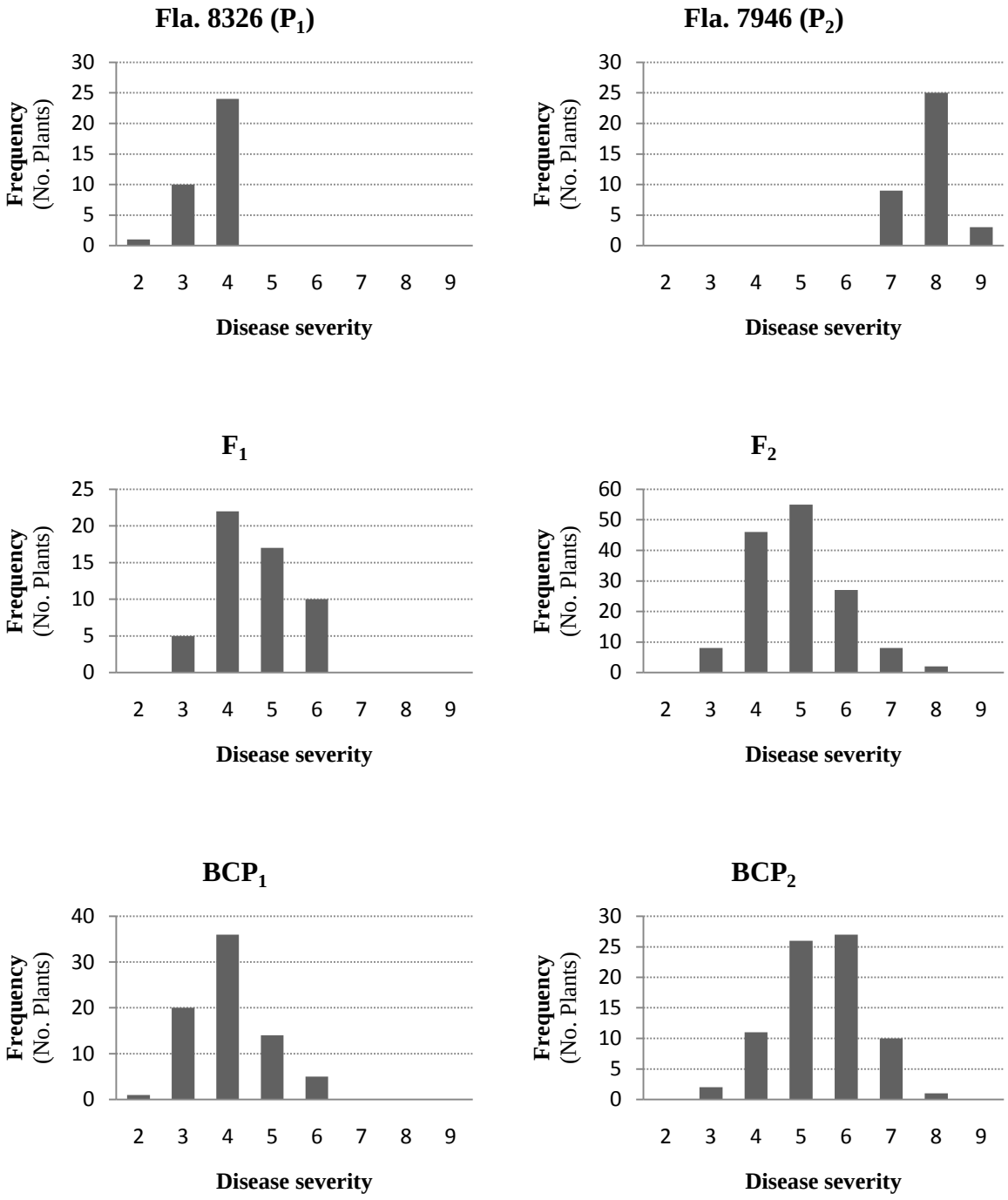


Figure 2-2. Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8326, Fla. 7946, and generations derived from them at Balm, FL, during spring 2006. BC = backcross. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text).

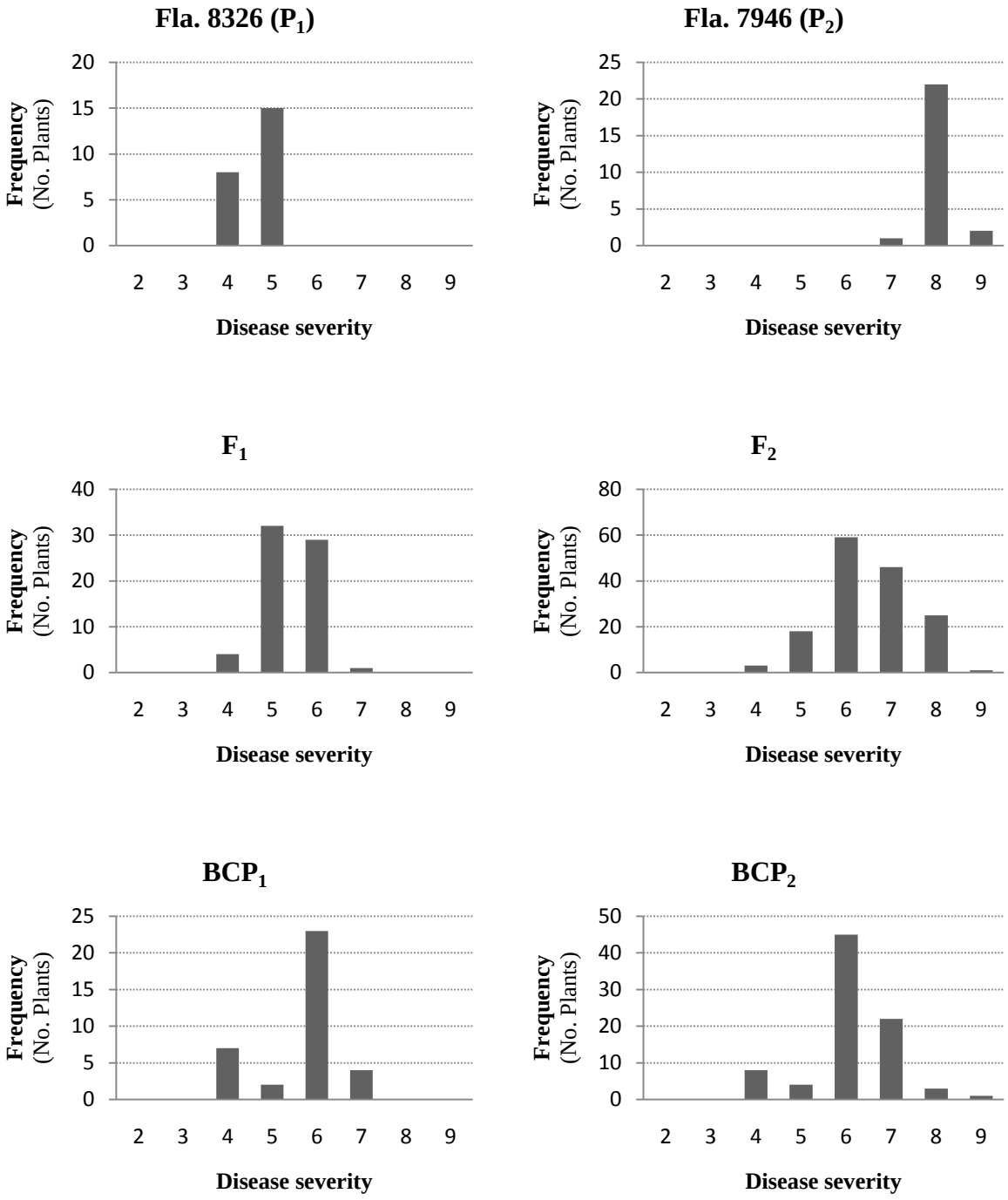


Figure 2-3. Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8326, Fla. 7946, and generations derived from them at Citra, FL, during summer 2007. BC = backcross. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text).

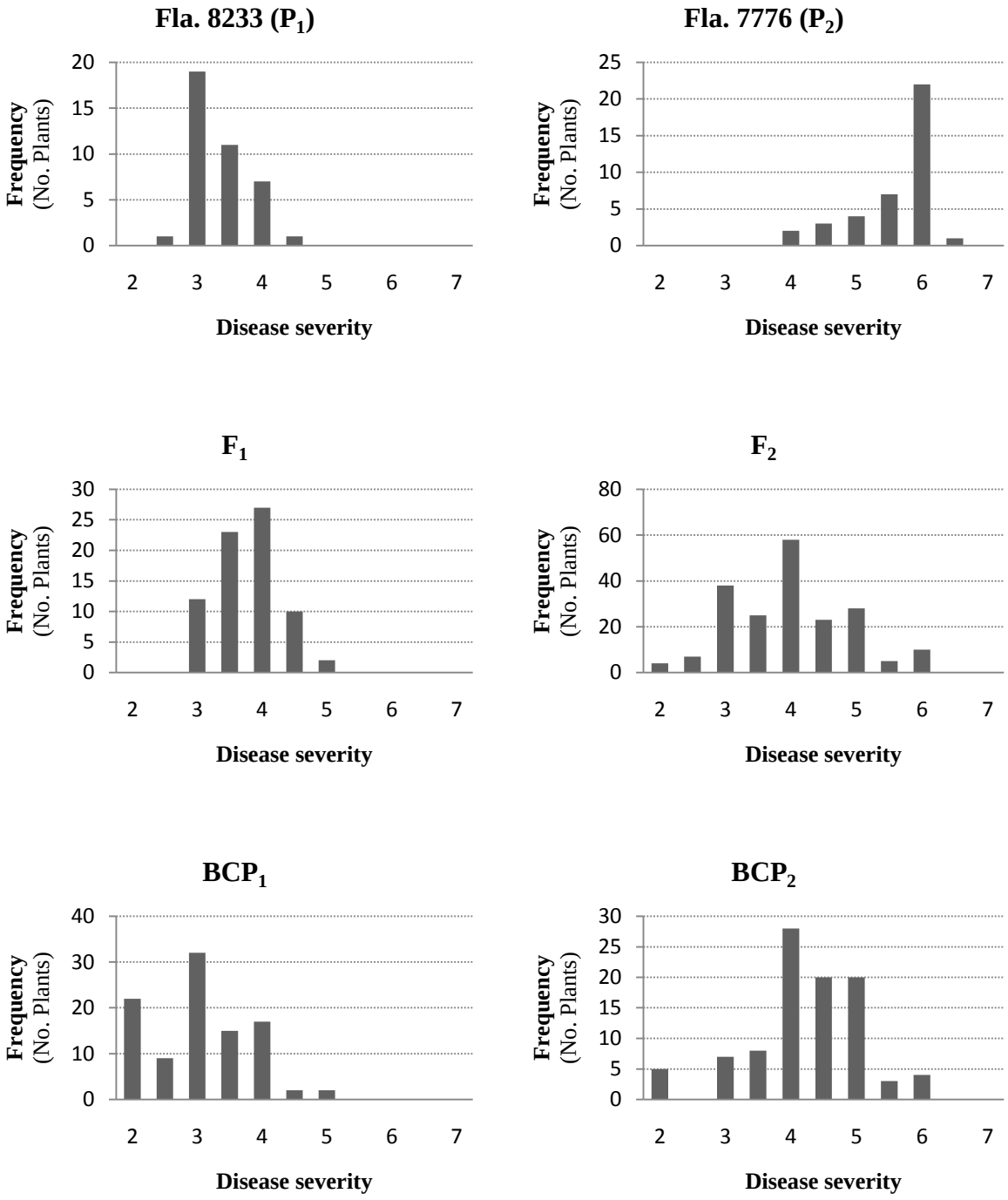


Figure 2-4. Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8233, Fla. 7776, and generations derived from them at Balm, FL, during spring 2007. BC = backcross. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text).

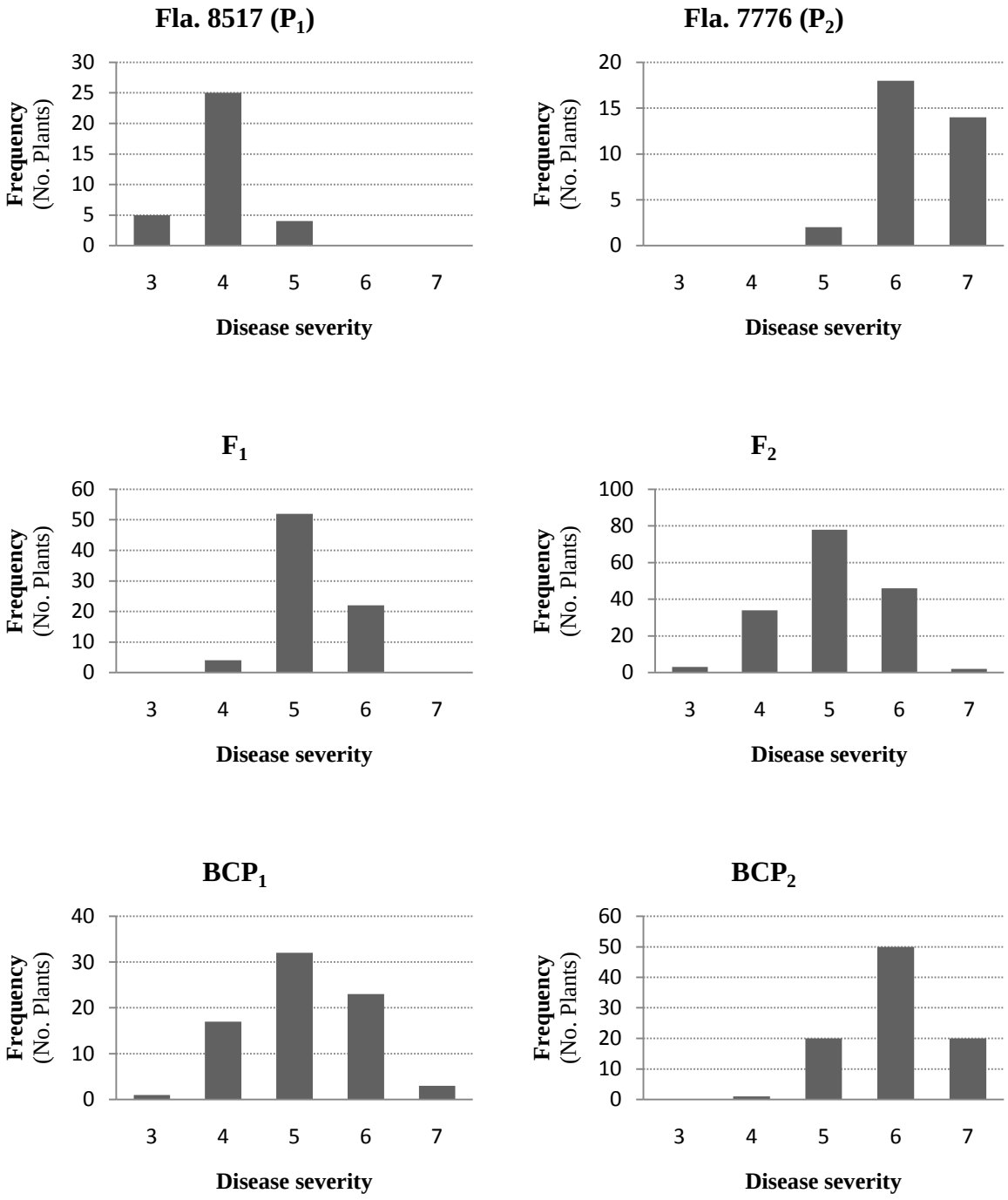


Figure 2-5. Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8517, Fla. 7776 and generations derived from them at Citra, FL, during summer 2007. BC = backcross. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text).

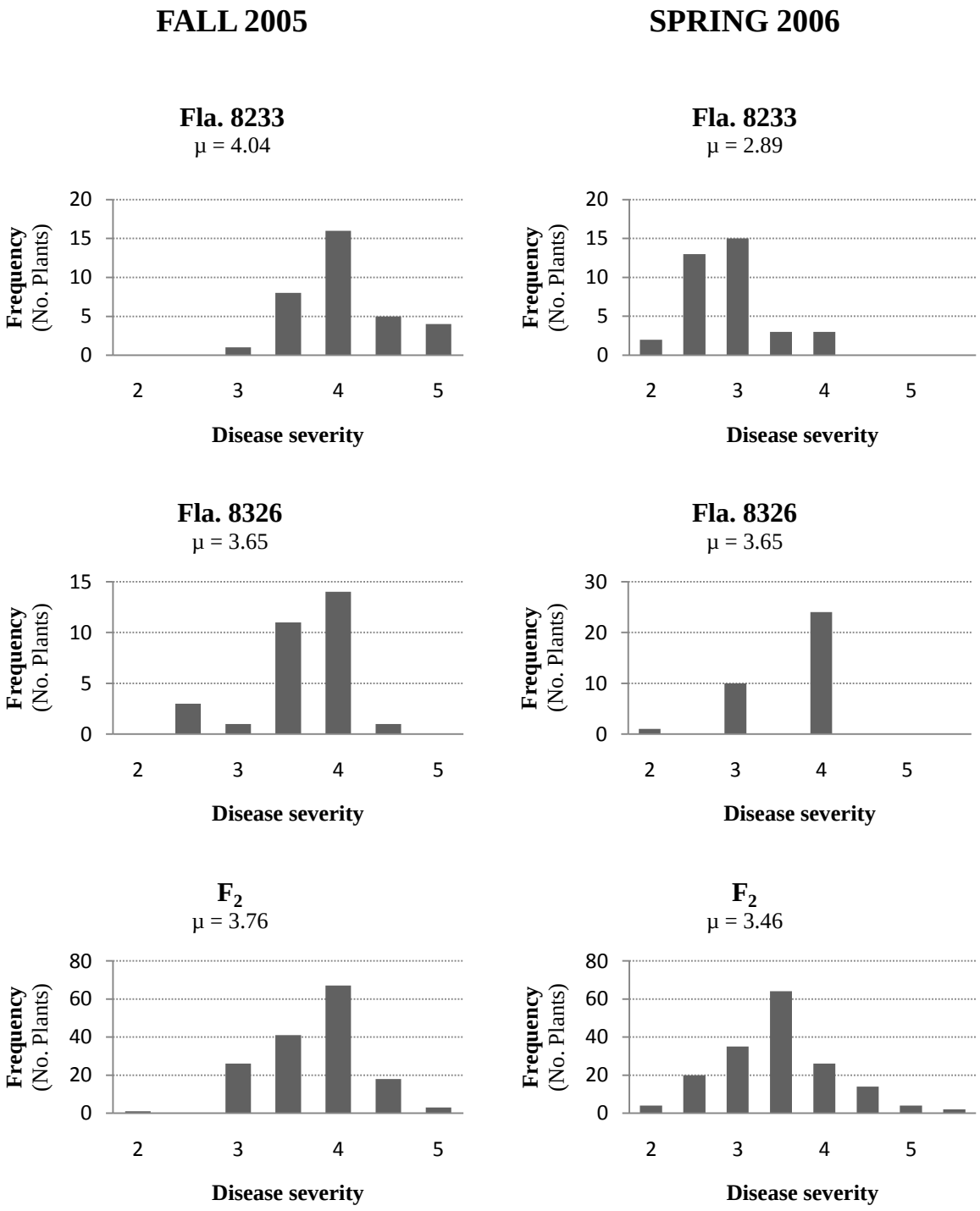


Figure 2-6. Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8233, Fla. 8326, and the F_2 generation derived from them at Citra, FL, during fall 2005 and spring 2006. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text).

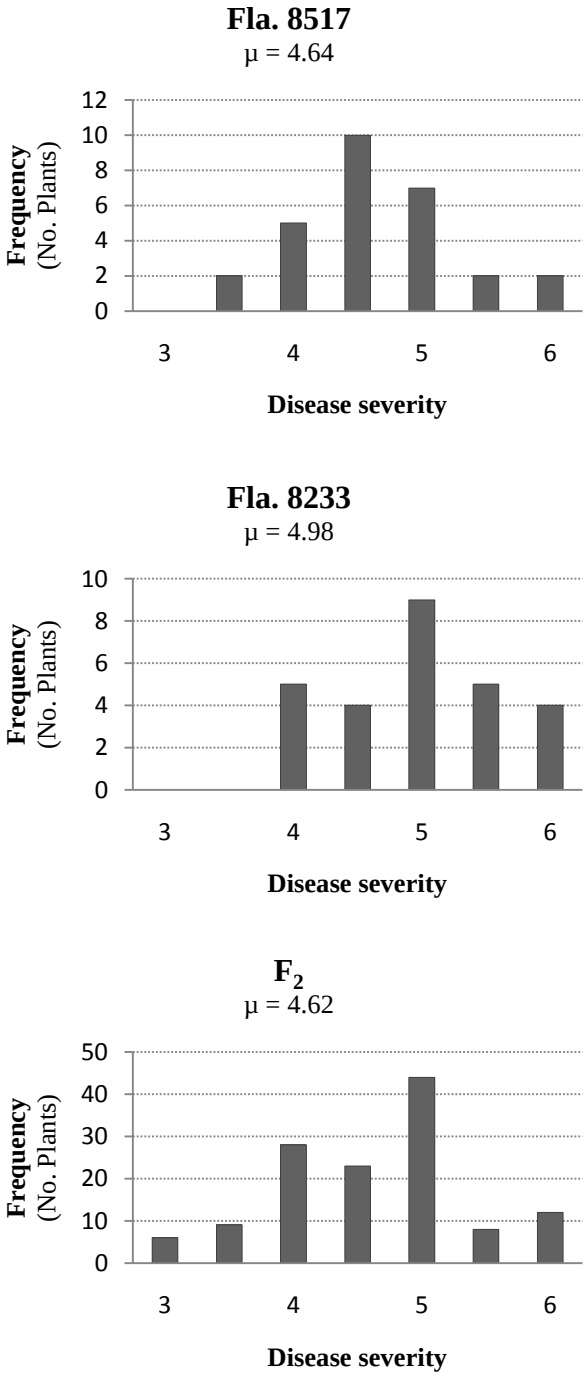


Figure 2-7. Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8517, Fla. 8233, and the F_2 generation derived from them at Citra, FL, during summer 2007. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text).

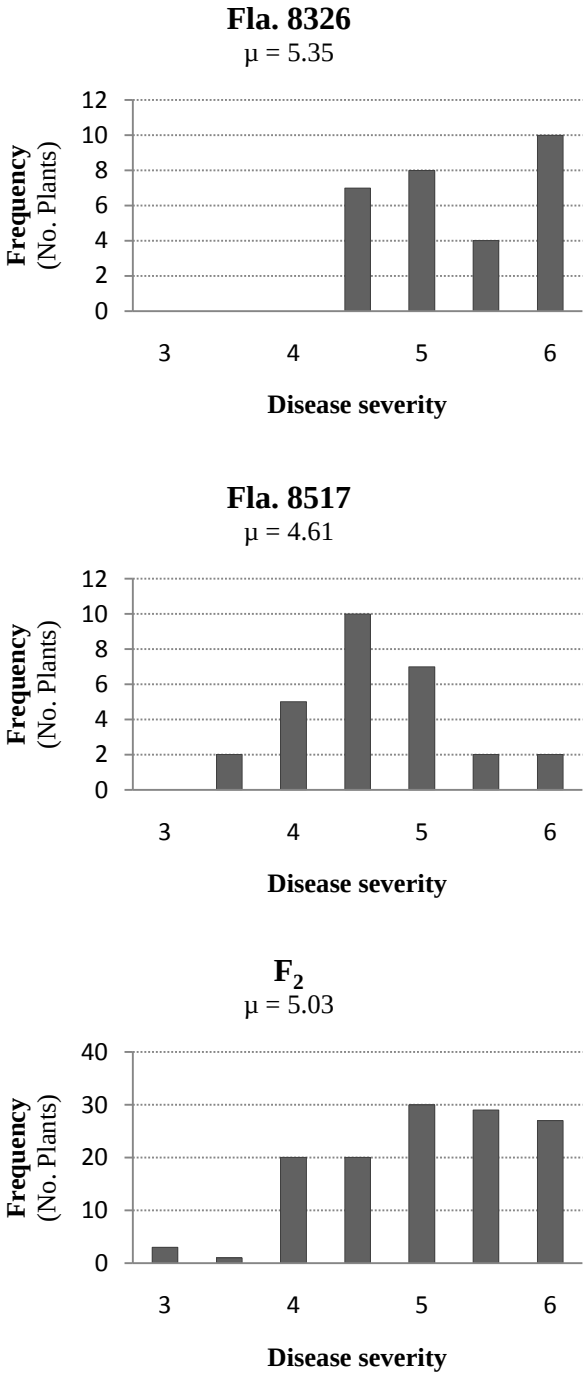


Figure 2-8. Bacterial spot race T4 disease severity frequency distributions for tomato parents Fla. 8326, Fla. 8517, and the F_2 generation derived from them at Citra, FL, during summer 2007. Plants were rated on the Horsfall-Barratt (1945) scale, where higher numbers indicate more disease (see text).

CHAPTER 3

ANALYSIS OF MOLECULAR MARKERS FOR LINKAGE TO RESISTANCE LOCI IN FLORIDA BREEDING LINES 8233, 8517 AND 8326

Introduction

Bacterial spot of tomato is the most pervasive disease that faces Florida tomato (*Solanum lycopersicum* L.) production. It is caused by three species of Xanthomonas: *Xanthomonas euvesicatoria, X. vesicatoria,* and *X. perforans* (Jones et al., 2000; Jones et al., 2006). Disease prevalence is greatest when temperatures are high and rainfall is frequent, conditions typically experienced in Florida during the early fall. Severe losses can occur due to leaf infections that cause defoliation, which may result in both yield losses and reduced quality from cracking, sunscald, or black shoulder (Scott and Jones, 1986).

Bacterial spot is primarily controlled by the use of bactericides which provide less than adequate control during periods of high disease pressure (Jones and Jones, 1985; Jones et al. 1991a, b). There has been much effort directed to breeding for resistant varieties, but development has been difficult due to the multigenic control of resistance and the emergence of new races of the pathogen. Prior to 1989, all strains that had been collected from various areas of the world were of a single race (T1) of *X. euvesicatoria*. This strain induced a hypersensitive response (HR) on the resistant genotype Hawaii 7998 (H7998) (Jones and Scott, 1986; Scott and Jones, 1986), and efforts were underway to incorporate this resistance into improved germplasm (Scott and Jones, 1989; Scott et al. 1991). Field resistance was reported to be largely additive and controlled by three to five effective factors, but was not explained by hypersensitivity alone (Scott and Jones, 1989; Wang, 1992; Somodi et al., 1996). Genetic control of the HR was initially determined to be associated with three regions of the genome: *Rx-1*, located on the short arm of chromosome 1; *Rx-2*, located on the long arm of chromosome 1; and *Rx-3*, located on

chromosome 5 (Yu et al., 1995). Subsequent work by Yang et al. (2005) using molecular markers linked to *Rx-1* and *Rx-3* determined that *Rx-3* was the primary contributor to race T1 HR, and that this locus explained 41% of field resistance to race T1 in an inbred backcross population.

In 1989, race 2 (T2) (*X. vesicatoria*) was determined to be quite prevalent in Brazil (Wang et al., 1990), and other areas of the world (Bouzar et al., 1994b; Stall et al., 1994). Strains of a third race (T3), *X. perforans*, which were classified originially as race T2, were isolated in Florida beginning in 1991 and were described by Jones et al. (1995). Race T3 strains induced a rapid hypersensitive response on the tomato genotype Hawaii 7981 (H7981) and *S. pimpinellifolium* accessions PI 126932 and PI 128216. In vitro studies found that race T3 was antagonistic to race T1 (El Morsy et al., 1994), and before race T1-resistant cultivars could be developed in Florida, the race T3 strain largely replaced T1 (Jones et al. 1998).

Scott et al. (1995) reported on the hypersensitivity and/or resistance of a number of lines to race T3 in 2 years of testing, including Hawaii 7981 (H7981), PI 128216, PI 126932, H7998 and PI 114490. The former three lines all produced a hypersensitive reaction when infiltrated with *X. perforans* race T3, whereas the latter two did not. Of the former three lines, H7981 showed the highest level of resistance, and selections of PI 126932 and PI 128216 had partial resistance. PI 114490 displayed partial resistance, and H7998 exhibited a low level of resistance. The hypersensitive response in H7981 was determined to be controlled by an incompletely dominant gene, *Xv3* (Scott et al., 1996), but field resistance was later determined to be quantitatively conferred by *Xv3* and other resistance genes (Scott et al., 2001).

Because of the potential for *X. vesicatoria* race T2 to emerge in Florida, resistance to this race, as well as to *X. euvesicatoria* race T1 and to *X. perforans* race T3 was desired. Scott et al.

(1997) screened a number of tomato genotypes for resistance to race T2 and compared these data to published results for races T1 and T3. H7981 was highly resistant to race T3 but susceptible to races T1 and T2. PI 126932 and PI 128216 were also resistant to race T3 but susceptible to races T1 and T2. H7998, resistant to race T1, had a low level of resistance to races T2 and T3. PI 114490 was one of only two lines with desirable levels of resistance to all three races, and had the highest and most consistent levels. Scott et al. (2003) determined that race T2 resistance in PI 114490 is additive and controlled by two genes; it was also reported that a strong relationship existed between resistance genes in this PI for races T1 and T2, but that race T3 resistance is likely controlled by different genes.

Recently a fourth race, *X. perforans* race T4, has emerged that overcame the *Xv3*-based hypersensitive resistance in H7981, PI 128216 and PI 126932 (Minsavage et al., 2003; Astua-Monge et al., 2000b). Scott et al. (2003) pointed out that a durable resistance that is effective across races is needed. In particular, since races T3 and T4 are the prevalent races in Florida, resistance to both of these races is needed to prevent bacterial spot. PI 114490 is resistant to race T4 as well as to the first three races of the pathogen (Scott et al., 2006), but little is known about the genetics of this resistance; furthermore, although race T4 overcame the T3 HR resistance of PI 128216 and PI 126932, the former has non-hypersensitive resistance to race T4, and breeding lines with race T4 resistance presumably derived from these PIs have been developed (Scott et al., 2006). The identification of molecular markers linked to resistance genes from each of these sources would be very useful in incorporating resistance into improved germplasm, as well as in efforts to pyramid resistance genes.

Three advanced breeding lines have been developed that have resistance to race T4. Florida 8233 is a large-fruited fresh market tomato with PI 114490 and H7998 recorded in its

pedigree. It has a moderate to high level of resistance to race T4. Florida 8517, with PI 114490, PI 128216 and H7998 in its pedigree, is a plum tomato with moderate to high resistance. Florida 8326 has PI 126932 and H7998 in its pedigree. It is a large-fruited fresh market tomato with only a moderate level of resistance. The primary objective of this research was to identify molecular markers linked to T4 resistance genes in each of these resistant breeding lines. A secondary objective was to develop and compile an inventory of markers polymorphic among resistant and susceptible genotypes used in this study.

Materials and Methods

Plant Materials

Three advanced breeding lines with resistance to bacterial spot race T4 of tomato were used as donor parents to develop three separate F_2 populations. Florida 8233, Fla. 8517 and Fla.8326 were selected for field resistance over multiple seasons, without using molecular markers. Fla. 8233 and Fla. 8517 were each crossed to Fla. 7776, a susceptible inbred line. Fla. 8326 was crossed to Fla. 7946, a highly susceptible inbred line. Individual F_2 plants, selected from each population on the basis of highest or lowest level of disease severity, were re-evaluated as F_3 (and in some cases, F_4) families to confirm resistance or susceptibility. Selections with consistently high or low levels of disease severity were genotyped with markers polymorphic between the two parents of their respective family. In screens to identify these polymorphic markers, PI 114490, PI 128216, PI 126932, H7981 and H7998 were included as potential resistance donors, and Fla. 7776 and Fla. 7946 were included as susceptible genotypes.

Molecular Markers

Approximately 500 PCR-based markers were screened to detect polymorphisms among PI 114490, PI 128216, PI 126932, H7981, H7998, Fla. 7776 and Fla. 7946. Many of the markers

were obtained from the Tomato Mapping Resource Database (http://www.tomatomap.net) or were recently developed (M. Robbins and S. Sim, personal communication), and had accompanying polymorphism information for genotypes used in this study. All other markers, unless otherwise indicated, were obtained from the *Solanaceae* genome network (SGN) (http://www.sgn.cornell.edu) or were developed in-house from RFLP probe, unigene or BAC sequences found on SGN.

For markers that did not have accompanying polymorphism information, screening was initially done to identify CAPS (cleaved amplified polymorphic sequence) markers. Here, PCR product of each marker was digested with 15 to 20 different frequent-cutting restriction enzymes to identify polymorphic restriction sites among the genotypes screened. Additional markers polymorphic between PI 114490 and Fla. 7776 were identified using an EcoTILLING approach on agarose gels, as described by Raghavan et al. (2007), with two exceptions: When making an enzyme mixture, 10x NEBuffer 1 (New England Biolabs, Inc.) was added to the celery juice extract (CJE) in CJE buffer and nanopure water, instead of the additional celery juice extract buffer; also digestions were carried out at 45˚C for one hour rather than for 15 minutes. Polymorphic markers identified by this approach were then sequenced and, where possible, converted to CAPS for screening of other genotypes. The EcoTILLING approach was also used to screen several polymorphic markers that could not be converted to CAPS.

Experimental Design, Inoculation and Disease Evaluation

Within each inheritance study, a randomized complete block design was used with four blocks, each with ten plants per plot for the parent lines and two plots of 25 plants for the F_2 generation. Progeny of F_2 selections were evaluated in confirming experiments, where a randomized complete block design was used with 3 blocks and 6 or 8 plants per plot. For all

experiments, seed were sown in growth rooms in Black Beauty spent coal (Reed Minerals Div., Highland, IN) and transplanted approximately 7 to 10 days later to Speedling® trays (3.8 cm^3 cell size) (Speedling, Sun City, FL) in the greenhouse, where seedlings were grown for four weeks. Plants were transplanted to field beds that were 20 cm high and 81 cm wide that had been fumigated with 67% methyl bromide : 33% chloropicrin at 197 kg ha^{-1} (175 lbs per acre) and covered with reflective plastic mulch. Plants were spaced 46 cm apart in rows, with 152 cm between rows, staked and tied, and irrigated by drip tape beneath the plastic mulch of each bed. A recommended fertilizer program was followed, and plants were sprayed with pesticides (excluding copper) as needed throughout the season (Olsen et al., 2007-2008).

Inoculum was produced by growing the bacteria on Difco nutrient agar (Becton Dickinson and Company, Sparks, Md.) for 24-36h at 28˚C. Bacterial cells were removed from the agar plates and suspended in 10 mM $MgSO_4 \cdot 7\ H_2O$, and the suspensions were standardized to A_{600} = 0.30 (a concentration of approximately 2 to 5 x 10^8 colony forming units (cfu)/mL). Inoculum was applied either at this concentration without surfactant, or was diluted to approximately 10^6 cfu/mL subsequent to standardization and applied along with Silwet L77 at 0.025% (v/v), as indicated below. Inoculum was applied by misting the foliage with a backpack sprayer. Plants were rated for disease severity in the field using the Horsfall and Barratt scale (1945), where 1 = 0%, 2 = 0%-3%, 3 = 3%-6%, 4 = 6%-12%, 5 = 12%-25%, 6 = 25%-50%, 7 = 50%-75%, 8 = 75%-87%, 9 = 87%-94%, 10 = 94%-97%, 11 = 97%-100%, and 12 = 100% diseased tissue.

Florida 8233

Florida 8233 was crossed to Fla. 7776, and subsequently the F_1 was self-pollinated to produce F_2 seed. Both parents and the F_2 generation were included in an inheritance study in fall

2006 and repeated in spring 2007 and summer 2007. In fall 2006, seed were sown on 17 July and transplanted to the field in Balm, FL on 25 August. Race T4 inoculum (at a concentration of approximately 2 to 5 $x10^8$ cfu mL^{-1}) was applied early in the morning of 20 September, and plants were rated for disease on 17 October. For the spring 2007 inheritance study, seed were sown on 1 February and transplanted to the field in Balm, FL on 13 March. Race T4 inoculum (same concentration as above) was applied on 25 April, and individual plants were rated for disease severity on 2 May. For the summer 2007 inheritance study, seed were sown on 8 June, and race T4 inoculum (at a concentration of approximately 2 to 5 $x10^6$ cfu mL^{-1}) was applied to plants on 20 July, prior to transplanting to the field in Citra, FL on 26 July. Individual F_2 plants were selected for resistance or susceptibility within each experiment.

Progeny of F_2 selections made in fall 2006 were evaluated in experiments in spring 2007 and summer 2007 to confirm resistance or susceptibility; progeny of spring 2007 selections were evaluated in a confirming experiment in summer 2007; and progeny of F_2 selections made in summer 2007 were evaluated in spring 2008. For the spring 2007 confirmation experiment in Balm, FL, plants were rated for disease severity on 1 May, and all other procedures were the same as described for the spring 2007 inheritance study, above. Likewise for the summer 2007 confirmation experiment in Citra, FL, disease severity was rated on 26 September, and all other procedures were the same as for the summer 2007 inheritance study. For the spring 2008 experiment in Balm, FL, low disease levels resulted in ambiguous data that will not be presented.

Florida 8517

Florida 8517 was crossed to Fla. 7776, and subsequently the F_1 was self-pollinated to produce F_2 seed. Both parents and the F_2 generation were included in an inheritance study in fall 2006 at Balm, FL and repeated in summer 2007 at Citra, FL. In fall 2006, plants were rated for

disease on 18 October, and in summer 2007 plants were rated on 3 October. All other procedures were the same as described for the Fla. 8233 inheritance studies.

Progeny of F_2 selections made in fall 2006 were evaluated in experiments in spring 2007 and summer 2007 to confirm resistance or susceptibility; and progeny of F_2 selections made in summer 2007 were evaluated in a confirming experiment in spring 2008. For the spring 2007 experiment, all procedures were the same as for the spring 2007 Fla. 8233 confirmation experiment above. Likewise for the summer 2007 confirmation experiment in Citra, FL, disease severity was rated on 26 September, and all other procedures were the same as for the Fla. 8233 summer 2007 inheritance study. In the spring 2008 experiment in Balm, FL, low disease levels resulted in ambiguous data that will not be presented.

Florida 8326

Florida 8326 was crossed to Fla. 7946, and subsequently the F_1 was self-pollinated to produce F_2 seed. Both parents and the F_2 generation were included in an inheritance study in spring 2006 and repeated in summer 2007. For the spring 2006 experiment, seed were sown on 17 February and transplanted to the field in Balm, FL on 29 March. Race T3 inoculum (at a concentration of approximately 2 to 5 x 10^8 cfu mL^{-1}) was applied to the plants early in the mornings on 5 May and again on 24 May because conditions were not favorable for disease during the first inoculation. However, subsequent race identification of plant lesions, and disease severity of control lines, indicated that race T4 caused the resultant field infection. Each plant was rated for race T4 disease severity the week of 26 June. For the summer 2007 inheritance study, each plant was rated for disease on 3 October; all other procedures were the same as described for the summer 2007 Fla. 8233 inheritance study.

Progeny of F_2 selections made in spring 2006 were evaluated in confirming experiments in spring 2007 and summer 2007, and progeny of F_2 selections made in summer 2007 were evaluated in spring 2008. For the spring 2007 experiment, all procedures were the same as for the spring 2007 Fla. 8233 confirmation experiment, above. Likewise for the summer 2007 confirmation experiment in Citra, FL, disease severity was rated on 26 September, and all other procedures were the same as for the Fla. 8233 summer 2007 inheritance study. In spring 2008, seed were sown on 5 February and transplanted to the field in Balm, FL on 13 March. Fields were naturally infected with race T4, and each plant was rated for disease on 2 May.

Marker Analysis

Within each family, markers polymorphic between the two parents were analyzed across selected progeny based on a modification of the Transmission Disequillibrium (TD) Test (George, et al., 1999; Zhu and Elston, 2001). For this approach, selections were grouped as resistant or susceptible, and marker data were scored on the basis of the probability of a resistant allele (++, +/-, --) for co-dominant markers, or on the basis of the presence of a resistant allele (+, -) for dominant markers. A regression analysis was used for all markers using the Reg procedure of the Statistical Analysis System (SAS Institute, Cary, N.C.).

Results

Two-hundred-five molecular markers without polymorphism information were initially screened by restriction digestions to identify polymorphisms among resistant and susceptible genotypes. Compared with Fla. 7776; 92 of these markers were polymorphic with PI 128216, 80 were polymorphic with PI 126932, and 19 were polymorphic with PI 114490. Because this latter PI was considered to be an important source of resistance in two of the resistant breeding lines, 227 markers (some of them previously screened by restriction digestions) were screened for

polymorphisms between PI 114490 and Fla. 7776 by the modified EcoTILLING approach (Table B-1). Thirty polymorphic markers were identified by this approach, 24 of which were included in the screening of resistant and susceptible genotypes. One hundred forty-two additional polymorphic markers were included on the basis of accompanying polymorphism information for a total of 269 markers polymorphic among resistant and susceptible genotypes (Table 3-1).

Florida 8233

Eleven resistant and five susceptible selections were made from the Fla. 8233 x Fla. 7776 F_2 generation over three seasons (Table 3-2). In fall 2006, E507-225 was selected as a resistant F_2 plant. Segregation for resistance was observed among its F_3 progeny in spring 2007, and two divergent selections were made, resulting in resistant selection 4 and susceptible selection 2. summer 2007 was the best season for distinguishing resistant and susceptible selections. Resistant selections 7 through 11 and susceptible selections 3 through 5 correspond to F_2 plants selected in summer 2007. Progeny of these later selections were included in a confirmation experiment in spring 2008, but differences between resistant and susceptible selections were not clear due to low disease pressure. Thus, inclusion of these selections in marker analysis is based solely on their F_2 ratings in summer 2007.

Twenty-one polymorphic markers were identified between Fla. 8233 and Fla. 7776 (Table 3-3), representing as many as 10 introgression regions. Three introgressions on chromosomes 4 (CT20145 to CT10184), 5 (CT20210I to Rx3-L1) and 9 (SSR383) appear to have originated from PI 128216, while no introgression in Fla.8233 necessarily originated from PI 114490. H7998 appears to be the source of an introgression on chromosome 11 (C2_At3g54470 to CT20181). The source of an introgression on chromosome 7 (CT20052 to

C2_At5g20180) could not be determined by its three representative markers. Five individual markers indentified possible introgression regions on chromosomes 1, 3, 7 and 10.

Analysis of markers for TD did not identify any marker as significant at the $P = 0.05$ level. One marker, TG403, was marginally non-significant ($P = 0.059$). The markers LEOH316 and Rx3-L1 on chromosome 5, although not significant, together indicate the possibility that they could be flanking a resistance locus because of the pattern of recombination between them. Chromosome 11 markers TG286-3 and CT20181 also show a pattern of resistance associated with the H7998 allele at this locus, with this allele present in several of the resistant selections and absent in all of the susceptible selections. Likewise, the chromosome 9 marker, SSR383, seems to show a pattern of resistance being associated with the Fla. 8233 allele, as several of the resistant selections were homozygous for this allele, but susceptible selections were all heterozygous or homozygous for the Fla. 7776 allele.

Florida 8517

Nine resistant and 8 susceptible selections were made from the Fla. 8517 x Fla. 7776 F_2 generation over two seasons (Table 3-4). In fall 2006, E514-246 was selected as a resistant F_2 plant. Segregation for resistance was observed among its F_3 progeny in spring 2007, and two divergent selections were made, resulting in resistant selection 1 and susceptible selection 3. Resistant selections 5 through 9 and susceptible selections 5 through 8 correspond to F_2 plants selected in summer 2007. Progeny of these selections were included in a confirmation experiment in spring 2008, but low disease pressure in the latter season, made it difficult to discern differences in resistant and susceptible selections. Thus, inclusion of these later selections is based solely on their F_2 ratings in summer 2007.

Forty-eight markers were polymorphic between Fla. 8517 and Fla. 7776, representing as many as 15 introgression regions in Fla. 8517 (Table 3-5). Introgressions on each of chromosomes 2, 3, 4 and 11 were initially found to have originated from either PI 114490 or PI 128216. Upon analysis of Fla. 8349 and Fla. 8350—the parents of Fla. 8517 (Figure A-1)—the introgressions on chromosomes 2 and 3 were determined to have descended from PI 114490 via Fla. 8350, the chromosome 4 introgression descended through Fla. 8350 and appears to have come from Fla. 7600, and one of the introgressions on chromosome 11 was determined to have originated from PI 128216 and descended through Fla. 8349 (data not shown). A second chromosome 11 introgression originated from either H7998 or PI 114490. The chromosome 5 introgression, originally thought to have come from PI 128216, appears to have resulted from a recombination event between markers Rx3-L1 and CosOH73. The upper portion of this introgression apparently originated from the processing line OH9242 in the pedigree of Fla. 8350, while the lower portion came from PI 128216 in the pedigree of Fla. 8349 (data not shown). The introgression on chromosome 12 also appears to have resulted from a recombination event within two separate introgressed regions in each of Fla. 8349 and Fla. 8350. The upper portion of this Fla. 8517 introgression (CT100 to C2_At5g42740) descended through Fla. 8349, likely from PI 128216, while the lower portion (SSR20) corresponds to the Fla. 7600/OH9242 allele from Fla. 8350 (data not shown). Six individual markers indentified possible introgressions on chromosomes 1, 7, 8, 9 and 10.

Three introgression regions were significant for TD in the Fla. 8517 family at the P = 0.05 level (Table 3-5). The chromosome 3 introgression from PI 114490 was a highly significant resistance locus for all four markers representing that region. The PI 128216 introgression on chromosome 11 was also a significant resistance locus for all three corresponding markers.

SSR20 on chromosome 12 was also highly significant, indicating that the Fla. 7600/OH9242 allele at this locus is associated with susceptibility. Although not significant, SSR383 on chromosome 9 showed a pattern of resistance associated with the Fla. 8517, as several of the resistant selections are homozygous for this allele, but susceptible selections are all heterozygous or homozygous for the Fla.7776 allele. Likewise, the pattern of recombination between markers CT10050 and CT10649, representing the chromosome 2 PI 114490 introgression in Fla. 8517, indicate the possibility that they could be flanking a resistance locus.

Florida 8326

Seven resistant and 8 susceptible selections were made from the Fla. 8326 x Fla. 7946 F_2 generation over two seasons (Table 3-6). In spring 2006, E707-166 was selected as a resistant F_2 plant. Segregation for resistance was observed among its F_3 progeny in spring 2007, and two divergent selections were made, resulting in resistant selection 7 and susceptible selection 1. Susceptible selection 4 segregated for resistance in summer 2007 and spring 2008. Despite the low disease pressure in spring 2008, a clear disease screen was achieved, confirming selections made the previous season; this was attributed to the high susceptibility of Fla. 7946, which resulted in sufficient contrast between resistant and susceptible selections.

Nineteen markers were polymorphic between Fla. 8326 and Fla. 7946, representing up to 9 regions of introgression in Fla. 8326 (Table 3-8). Three markers on chromosome 3 as well as two individual markers on chromosome one and TG403 on chromosome 10 indicated introgressions that likely descended from PI 126932. Eight markers on chromosome 11 represent an introgression from H7998. Five additional markers indicated possible introgressions on chromosomes 2, 3, 7 and 9.

All chromosome 11 markers were significant for TD at the $P = 0.05$ level in the Fla. 8326 family, while markers TOM196, SSR637, TOM144 and LEOH57 were highly significant ($P < 0.0001$). No other regions of introgression were significant, although marker Cf9 on chromosome 1 was marginally non-signficant ($P = 0.063$).

Discussion

The rapid rate at which resistance to bacterial spot of tomato has been overcome is quite alarming, especially when considering that this has occurred without the deployment of resistant cultivars. For resistance to be successful, it must be effective against all present races of the pathogen and durable against the emergence of new races. Our approach has been to utilize molecular markers to identify resistance genes from a number of different sources conferring both race-specific and broad spectrum resistance, and then to pyramid these genes to possibly attain a higher level of resistance. PI 114490, PI 128216, PI 126932 and H7998 each have resistance or partial resistance to multiple races of bacterial spot. Florida breeding lines 8233, 8517 and 8326 also have resistance to multiple races of bacterial spot (Scott et al., 2006), presumably derived from one or more of these sources.

Despite the pervasiveness of bacterial spot in Florida, periods of lower disease pressure often occur, especially in the spring production season when rainfall is less frequent. When disease pressure and secondary spread are low, the ability to distinguish resistant and susceptible genotypes depends on the level of contrast between the two. This was evidenced in the spring 2008 confirmation experiments: Florida 8326 has only moderate resistance to race T4, but progeny of F_2 selections were clearly distinguishable because of the high susceptibility of Fla. 7946; alternatively, because Fla. 7776 is not as susceptible as Fla. 7946, it was not possible to

clearly distinguish selections from the Fla. 8233 or Fla. 8517 families, even though each of these lines are more resistant than Fla. 8326.

Florida 8233 has PI 114490 recorded in its pedigree, but none of its introgressions identified by markers from this research were clearly descended from this PI. However, three (on chromosomes 4, 5 and 9) appear to have descended from PI 128216. It was already known that an error existed in the pedigree of Fla. 8233, because this line has T3 hypersensitivity, while none of the parents in its recorded pedigree are T3 hypersensitive (J. Scott, personal communication). These results suggest that the mistake most likely occurred when crossing Fla. 7655 with PI 114490 (see Figure A-2), and pollen from PI 128216 was used instead. By this explanation, PI 128216 would also be the source of the T3 hypersensitivity.

The lack of significance for markers in the Fla. 8233 family is likely due to the low number of individuals represented in the susceptible pool. Still, four regions of introgression showed a pattern of resistance associated with the Fla. 8233 allele. A PI 128216 introgression on chromosome 9 is present in both Fla. 8233 and Fla. 8517. While analysis of this marker for either family was non-significant, a combined analysis on selections from both families was significant ($P = 0.0217$). Although this PI 128216 allele was distributed among both resistant and susceptible selections, selections homozygous for this allele were only present within the resistant groups, suggesting that this locus may confer the additive resistance identified in each of these breeding lines by the generation means analysis (see Ch. 2). Similarly, marker TG403 on chromosome 10 failed to show significance in the Fla. 8233 or Fla. 8517 families. In this case, resistance appears to be associated with the PI 128216 allele in Fla. 8233 ($P = 0.059$), where only one susceptible selection is heterozygous and all others are homozygous for the Fla. 7776 allele, but resistance does not appear to be associated with the Fla. 8517 allele ($P = 0.488$). A

combined analysis on selections from both families was not significant ($P = 0.7841$). However, it could not be determined whether the Fla. 8517 allele descended from PI 128216 or from PI 114490. Thus, the possibility of a PI 128216 resistance QTL on chromosome 10 is not precluded.

Because the map positions for the markers used in this study were not all determined from a common mapping population, markers representing each introgression were arranged in the most logical order, considering the recombination patterns among selected progeny. Provided the orders are correct, the chromosome 5 region between markers LEOH316 and Rx3-L1 displayed a surprisingly high amount of recombination, with 20 of the 33 selections from Fla. 8233 and Fla. 8517 showing cross-overs in this region. In the Fla. 8233 family, 8 of the resistant selections are homozygous for the resistant allele for marker LEOH316, and no resistant alleles are present in susceptible progeny for marker Rx3-L1, suggesting that a resistance gene could be located between the two markers. Moreover, resistant selection 4 has at least one copy of the resistant allele at this locus, but susceptible selection 2 (selected divergently from the same F_3 plant as resistant selection 4) only has the susceptible allele. Such a pattern of association between observed resistance and the presence of the resistant allele was not observed in the Fla. 8517 family. However, the PI 128216 introgression in Fla. 8517 does not span the region between LEOH 316 and Rx3-L1, and thus does not include this plausible resistance locus. Having a resistance gene from PI 128216 at this locus seems possible, given that resistance genes are often organized in clusters, and this region contains a gene important for T1 hypersensitivity in H7998 (Yang, et al., 2005; Yu et al., 1995). A gene cluster at this locus could also explain the high rate of recombination within selected progeny, as varying levels of recombination are observed between component genes of a cluster (Michelmore and Meyers, 1998). Alternatively, a T4 resistance gene at this locus could simply be an alternative allele from that of H7998.

Both Fla. 8233 and Fla. 8326 have a chromosome 11 introgression from H7998. While the size of the introgression in Fla. 8326 appears to span a larger region, this is not necessarily the case. Instead, this could be due to the lack of polymorphic markers in the region that distinguish H7998 alleles from Fla. 7776 alleles. This is rather unfortunate, since the significance levels of chromosome 11 markers in the Fla. 8326 family indicate the QTL may be near the upper portion of this introgression, precisely where markers are lacking in the Fla. 8233 family. An obvious question with regard to this introgression is why such a clear effect is observed in the Fla. 8326 family, where all resistant selections possess the H7998 allele, while not all resistant selections in the Fla. 8233 family carry it. One possible explanation is that the upper part of the H7998 introgression containing the QTL is not present in Fla. 8233, and resistance in this line is not associated with this locus. The present research cannot rule out this possibility, especially considering the lack of significance for TD at this locus. On the other hand, those resistant selections in the Fla. 8233 family that appear to lack the introgression could be recombinants that only lost the lower portion of the introgression, while retaining the portion of the introgression for which there are no polymorphic markers. Another, very likely explanation is that resistance in Fla. 8233 may be quantitative, while Fla. 8326 may have only one major resistance gene. Thus, Fla. 8233 selections lacking a gene on chromosome 11 but possessing one or more genes from other loci, still show resistance relative to the moderately susceptible Fla. 7776; whereas Fla. 8326 selections must all carry the chromosome 11 QTL to express resistance relative to the highly susceptible Fla. 7946. This seems possible, as plausible QTL on chromosomes 5, 9 and 10 explain the resistance observed in Fla. 8233 resistant selections 1, 2, 4, 5, 6 and 9, which all lack the chromosome 11 introgression. Together with the chromosome 11 locus, these four QTL account for the resistance or susceptibility observed in 15 of the 16 Fla. 8233 selections. The lack

of resistance in susceptible selection 5, which has the resistant allele for marker TG403, could be explained by a possible recombination between the QTL and the marker, but more markers in this region would be needed to confirm this.

A problem with attributing T4 resistance in Fla. 8326 to a single H7998 gene is that Fla. 8326 exhibits a higher level of resistance to T4 than does H7998 (Scott et al., 2006). This is suggestive of epistasis, where some non-H7998 gene in Fla. 8326 is effecting this higher level of resistance from the chromosome 11 QTL. Indeed, only one factor was indicated in Fla. 8326 by the fall 2005 inheritance study (see Ch. 2); under the low disease pressure that season, the H7998 gene evidently provided sufficient resistance to explain the variation that was observed. However, under the higher disease pressure in spring 2006 and summer 2007, epistatic effects were significant, indicating that a second gene was required for full resistance.

The epistatic gene of Fla. 8326 would theoretically be present in resistant selections and absent in the susceptible selections that carried the chromosome 11 introgression. Marker Cf9 maps to a region on the short arm of chromosome 1 that was identified as a contributor to T1 hypersensitivity from H7998 (Yu et al., 1995). This marker indicates a PI 126932 introgression in Fla. 8326 that was marginally non-significant for TD analysis, but is a likely candidate for such a QTL. Resistant selection 4 is the only resistant individual that lacks this allele, but it also had one of the highest disease severity ratings of all resistant selections in summer 2007 (selection 7 was rated higher, but was also heterozygous for the chromosome 11 introgression). Of the susceptible selections that have the Cf9 resistant allele, only selection 4 carried the chromosome 11 introgression, and this selection also segregated for resistance. Moreover, susceptible selection 1 and resistant selection 7 were divergent selections from the same F_3 family, but only the latter carries the chromosome 1 resistant allele. Thus, this chromosome 1

locus seems to account for the recessive suppression type epistatic effect identified by the generation means analysis in Fla. 8326 (see Ch. 2). The same effect was likewise identified in Fla. 8233 and Fla. 8517 by their inheritance studies (see Ch. 2), and the chromosome 1 resistant allele is also present in most of the resistant selections from each of these families; however, the lack of susceptible selections missing this allele while having the chromosome 11 QTL limits the confidence with which the recessive suppression interaction can be inferred regarding Fla. 8233 and Fla. 8517. The development of one or more codominant markers in this region, together with the screening of larger resistant and susceptible pools, would help to confirm or rule out this possibility.

Hawaii 7997 is a source of resistance to bacterial wilt (*Ralstonia solanacearum*) in the University of Florida tomato breeding program, and it is susceptible to race T4 bacterial spot (J.W. Scott, personal comm.). This resistance has been incorporated into several breeding lines that are also susceptible to bacterial spot, yet resistance to bacterial spot race T4 has been observed in a number of advanced breeding lines developed from this material (Scott, unpublished). This is suggestive of an interaction either between a bacterial spot resistance gene in H7997 and an effecting gene in some of the susceptible breeding lines, or between a bacterial spot resistance gene in some of the susceptible breeding lines and an effecting gene in H7997. Both possibilities lend support to the present theory that epistasis is contributing to bacterial spot resistance, and that the native background of the resistance gene does not always contain the secondary gene.

The chromosome 12 introgression in Fla. 8517 apparently came about by a recombination event between a PI 128216 introgression in Fla. 8349 and an OH9242 introgression in Fla. 8350, as both introgressions were present in early selections of this breeding

line. The latter introgression, represented by SSR20, was a significant susceptibility QTL in the Fla. 8517 family, emphasizing that it is often an invalid assumption that resistant alleles are only present in the resistant parent. The association of this locus with susceptibility from OH9242 is supported by the fact that this allele was segregating in the early Fla. 8517 selection used in this study, but is absent in later selections of the same line (data not shown). QTL for resistance in Fla. 8517 are located on chromosomes 3, 9 and 11. The QTL on chromosomes 3 and 11 appear to exhibit dominant gene action and may be the primary contributors to the dominant effect identified by the generation means analysis (see Ch. 2). PI 114490 is the donor of the resistant allele on chromosome 3; this region has also been associated with the *Xv4*-based resistance to race T4 from *S. pennellii* LA 716 (Astua-Monge et al., 2000a). When Fla. 8517 was selected from the Fla. 8349 x Fla. 8350 F_2 plot, two additional selections were also made. Evaluation of the F_3 families from each of these selections indicated that the other two lines were less resistant than Fla. 8517. Interestingly, neither of these two additional selections carried the chromosome 3 introgression from PI 114490 or the chromosome 9 introgression from PI 128216. None of the other resistance loci demonstrated this pattern among the three selections (data not shown). PI 128216 is also the donor of the resistant allele on chromosome 11. If the PI 128216 chromosome 11 QTL is an allele at the same locus as in H7998, this research would suggest an allelic series where H7998 and PI 128216 > Fla. 7776 > Fla. 7946. The plausible Fla. 8517 resistance QTL on chromosomes 3, 9 and 11, and the susceptibility QTL on chromosome 12, account for the resistance or susceptibility observed in 16 of the 17 selections. Only resistant selection 1 also lacks all of the resistance QTL markers, but it also does not have the OH9242 susceptibility allele on chromosome 12.

The identification of multiple QTL conferring bacterial spot race T4 resistance from four separate sources raises hopes that an acceptable level of durable resistance can be achieved. The potential for successfully pyramiding these QTL to result in higher and more durable levels of resistance depends in part on whether the resistance genes have different mechanisms of resistance. Further research is necessary to determine whether these QTL contain unique resistance genes, or if they are essentially mimic genes. Efforts are underway both to pyramid these resistance QTL for evaluation of their combined effect, and to develop additional molecular markers for finer mapping and individual confirmation of each plausible resistance locus. Upon confirmation of all QTL, each locus should be tested to determine the level of resistance provided against each race of bacterial spot, as well as its mechanism of resistance. To aid in the evaluation of individual QTL, crosses have been made between Fla. 7946 and both Fla. 8233 and Fla. 8517 for QTL evaluation in a more susceptible background. Plausible resistance QTL are also being incorporated into a number of susceptible breeding lines by MAS using the markers identified by this research.

Table 3-1. Markers polymorphic among genotypes resistant or susceptible to bacterial spot.

		Allele												
Marker	Approximate position[z]	Fla.7776	Fla.7946	PI114490	PI128216	PI126932	H7981	H7998	Fla.8233	Fla.8517	Fla.8326	Marker type	Restriction enzyme	Reference source for primers
SSR478	1.000	1	2	2	1	2	2	2	2	2	2	SSR	n/a	SGN
Cf9	1.005	1	1	2	2	2	1	1	1	1	2	CAPS	*Hae* III	Tomatomap.net
CosOH47	1.010	1	1	1	2	2	1	1	1	1	1	CAPS	*Bst* UI	Tomatomap.net
LEOH36	1.019	1	1	1	2	2	2	2	1	1	1	CAPS	*Bcl* I	Tomatomap.net
C01HBa0003D15.1	1.029	1	1	1	1	2	1	1	1	1	1	CAPS	*Alu* I	Present
C2_At5g18580	1.035	1	1	1	1	2	1	1	1	1	1	CAPS	*Hpy* CH4IV	SGN
C2_At5g18580	1.035	1	1	2	2	2	1	2	1	1	1	CAPS	*Dde* I	SGN
CT20134I	1.041	1	1	1	2	3	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT10975I	1.051	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT10030I.1	1.058	1	1	2	1,2	1	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT20116	1.059	1	1	2	2	1	1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
CT10811	1.065	1	1	2	2	2	1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
CT10629	1.065	1	1	2	2	2	1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
CT10945	1.065	1	1	2	2	2	1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
SSR134	1.075	1	1	2	2	2	1	3	1	1	1	SSR	n/a	SGN
TOM202	1.082	1	1	2	3	4	1	1	1		1	SSR	n/a	Tomatomap.net
C2_At3g04710	1.095	1	1	1	2	2	1	1	1	1	1	CAPS	*Hinc* II	SGN
LEOH106	1.095	1	1	2	2	1	1	1	1	1	1	CAPS	*Alu* I	Tomatomap.net
TG59	1.097	1	1	2	2	2	1	1	1	1	1	CAPS	*Dpn* II	Present
TG59	1.097	1	1	1	1	1	2	2	1	1	1	CAPS	*Mnl* I	Present
LEVCOH11	1.100	1	1	2	2	2	1	1	1	1	1	CAPS	*Mnl* I	Tomatomap.net
LEVCOH12	1.101	1	1	2	2	2	1	1	1	1	1	CAPS	*Bsa* J I	Tomatomap.net
C2_At3g04870	1.102	1	1	1	2	1	1	1	1	1	1	CAPS	*Hpy* CH4IV	SGN
U237757	1.102	1	1	1	1	2	1	2	2	1	1	CAPS	*Mnl* I	SGN
C2_At1g02560	1.116	1	1	1	2	2	1	1	1	1	2	SCAR	n/a	SGN
SSR308	1.116	1	1	1	2	1	1	1	1	1	1	SSR	n/a	SGN
C2_At5g49880	1.127	1	1	1	2	1	1	1	1	1	1	CAPS	*Rsa* I	SGN
LE_HBa0044E20	1.128	1	1	1	2	1	1	1	1	1	1	CAPS	*Hpy* CH4IV	Present
C2_At4g14110	1.136	1	1	1	2	1	1	1	1	1	1	CAPS	*Hinc* II	SGN
SSR65	1.153	1	1	1	2	2	1	1	1	1	1	SSR	n/a	SGN

Table 3-1. Continued

Marker	Approximate position[z]	Allele										Marker type	Restriction enzyme	Reference source for primers
		Fla.7776	Fla.7946	PI114490	PI128216	PI126932	H7981	H7998	Fla.8233	Fla.8517	Fla.8326			
LEOH342	2.000	1	1	2	2		1	1	1	1	1	SCAR	n/a	Tomatomap.net
TOM11	2.014	1	1	2	1		1	2	1	1	1	SSR	n/a	Tomatomap.net
CT10682I	2.029	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
SSR66	2.031	1		2	2	1	1	1	1	1		SSR	n/a	SGN
SSR104	2.037	1	1	2	2	1	1	1	1	2	1	SSR	n/a	SGN
SSR96	2.039	1	1	2	2	1	1	1	1	2	1	SSR	n/a	SGN
LEOH23.3	2.042	1	1	2	2	1	1	1	1	2	1	CAPS	*Tsp* 509I	Tomatomap.net
CT10649	2.042	1	1	2	2	1	1	1	1	2	2	SNP	n/a	Sung-Chur Sim, personal comm.
SSR5	2.044	1	1	2	2	3	1	1	1	2	1	SSR	n/a	SGN
SSR349A	2.044	1	1	2	2	3	1	1	1	2	1	SSR	n/a	SGN
CT10923	2.044	1		2	2	2	1	1	1	2		SNP	n/a	Sung-Chur Sim, personal comm.
CT10153	2.048	1	2	2	2	1	2	1	1	2	2	SNP	n/a	Sung-Chur Sim, personal comm.
CT10771	2.048	1	2	2	2	1	2	1	1	2	2	SNP	n/a	Sung-Chur Sim, personal comm.
CT10801	2.051	1	1	2	2	1	1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
CT10279I	2.055	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
TOM188	2.059	1	1	2	2	3	1	1	1	2	1	SSR	n/a	Tomatomap.net
LEOH348	2.072	1	1	1	2	2	1	1	1	1	1	CAPS	*Hpy* CH4IV	Tomatomap.net
CosOH7	2.074	1	1	2	2	2	1	1	1	1	1	CAPS	*Hinf* I	Tomatomap.net
HBa44O16SP6	2.075	1	1	1	1	2	1	1	1	1	1	CAPS	*Hae* III	SGN
C2_At4g35560	2.079	1	1	1	2	1	1	1	1	1	1	CAPS	*Tsp* 509I	SGN
C2_At5g66090	2.083	1	1	1	2	1	1	1	1	1	1	CAPS	*Rsa* I	SGN
C2_At5g66090	2.083	1	1	1	2	2	1	1	1	1	1	CAPS	*Hpy* CH4III	SGN
LEOH319	2.107	1	1	2	2	2	1	1	1	1	1	CAPS	*Tsp* 509I	Tomatomap.net

Table 3-1. Continued

Marker	Approximate position[z]	Allele										Marker type	Restriction enzyme	Reference source for primers
		Fla.7776	Fla.7946	PI114490	PI128216	PI126932	H7981	H7998	Fla.8233	Fla.8517	Fla.8326			
CT10690I	3.003	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT10772I	3.004	1		2	2	2			1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT20050	3.004	1	1	2	2	2	1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
CT20182I	3.013	1		2	1	1			1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT20182	3.013	1	1	2	3	4			1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT10480I	3.038	1	1	2	2	2	2	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
LEOH223	3.040	1	1	2	2	2	1	1	1	1	1	CAPS	*Mse* I	Tomatomap.net
CT10402I	3.046	1	1	2			1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT20195	3.046	1	1	2	2	2	1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
T1388	3.047	1	1	2	2	2	1	2	1	1	1	CAPS	*Mnl* I	SGN
T1388	3.047	1	1	1	1	2	1	1	1	1	1	CAPS	*Hpy* CH4IV	SGN
CT20037	3.047	1	1	2	2	2	1	1	1	2	1	SNP	n/a	Sung-Chur Sim, personal comm.
CT10736	3.050	1	1	2	2	2	1	1	1	2	1	SNP	n/a	Sung-Chur Sim, personal comm.
LEOH124SNP	3.059	1	1	1	1	2	1	1	1	1	1	CAPS	*Hha* I	Tomatomap.net
LEGTOM5c	3.064	1	1	2	1	1	1	1	1	1	1	CAPS	*Mse* I	Tomatomap.net
SSR111	3.070	1	1	1	1	1	1	2	3	1	3	SSR	n/a	SGN
C2_At1g02140	3.071	1	1	2	2	2	1	1	1	2	1	CAPS	*Hha* I	SGN
SSR231	3.075	1	1	2	2	2	2	2	1	1	1	SSR	n/a	SGN
C2_At5g62390	3.076	1	1	2	2	2	1	1	1	2	1	CAPS	*Hinf* I	SGN
LEOH185	3.078	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	Tomatomap.net
FEY	3.079	1	1	2	2	2	1	1	1	1	1	CAPS	*Bst* UI	SGN
C2_At5g63460	3.083	1	1	1	2	2	1	1	1	1	1	CAPS	*Alu* I	SGN
C2_At5g60160	3.083	1	1	1	2	2	1	1	1	1	1	CAPS	*Hinf* I	SGN
C2_At1g05350	3.085	1	1	1	2	2	1	2	1	1	1	CAPS	*Dde* I	SGN
C03HBa0082F22	3.087	1	1	2	2	2	2	2	1	1	1	CAPS	*Hinc* II	Present
C2_At5g52820	3.090	1	1	1	2	2	1	2	1	1	1	CAPS	*Hpy* CH4IV	SGN
U146899	3.097	1	1	1	2	2	1	2	1	1	1	CAPS	*Hinc* II	SGN
C2_At5g49970	3.100	1	1	1	2	2	1	1	1	1	1	CAPS	*Rsa* I	SGN
C2_At3g47990	3.102	1	1	1	2	2	1	1	1	1	1	CAPS	*Aci* I	SGN
C2_At1g61620	3.106	1	1	2	2	2	1	1	1	1	1	CAPS	*Taq* I	SGN
CosOH51	3.107	1	1	2	1	1	2	2	1	1	1	CAPS	*Rsa* I	Tomatomap.net
SSR320	3.112	1	1	2	3	3	3	1	1	1	1	SSR	n/a	SGN
SSR601	3.112	1	1	2	3	4	2	1	1	1	1	SSR	n/a	SGN
LEOH127	3.113	1	1	2	2	2	1	1	1	1	1	CAPS	*Hinc* II	Tomatomap.net
CT10494	3.113	1	1	2	1	1	2	1,2	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.

Table 3-1. Continued

Marker	Approximate position[z]	Allele										Marker type	Restriction enzyme	Reference source for primers
		Fla.7776	Fla.7946	PI114490	PI128216	PI126932	H7981	H7998	Fla.8233	Fla.8517	Fla.8326			
Hero	4.007	1	1	1	1	2	1	1	1	1	1	CAPS	*Mnl* I	SGN
Hero	4.007	1	1	1	2	1	1	1	1	1	1	CAPS	*Mwo* I	SGN
TG15-2	4.012	1	1	1	2	2	1	1	1	1	1	CAPS	*Hpy* CH4IV	Present
TOM194	4.014	1	1	2	1	1	1	1	1	1	1	SSR	n/a	Tomatomap.net
SSR43	4.015	1	1	2	2	3	1	2	1	1	1	SSR	n/a	SGN
C2_At3g17040	4.022	1	1	1	2	2	1	2	1	1	1	CAPS	*Dde* I	SGN
CT20145	4.041	1	1	1	2	2	2		2	2	2	SNP	n/a	Sung-Chur Sim, personal comm.
CT188-2	4.042	1	1	1	2	1	1	1	1	1	1	CAPS	*Hha* I	Present
TG182	4.046	1	1	1	2	2	2	2	1	1	1	CAPS	*Rsa* I	SGN
CT10184	4.047	1	1	1	2	2	2	1	2	2	2	SNP	n/a	Sung-Chur Sim, personal comm.
C2_At5g37360	4.056	1	2	2	1	1	1	1	2	2	2	CAPS	*Mae* III	SGN
LEOH37	4.068	1	1	1	2	1	1	1	1	1	1	CAPS	*Tsp* 45I	Tomatomap.net
C2_At1g71810	4.072	1	1	1	2	2	1	1	1	1	1	CAPS	*Bst* UI	SGN
CT185	4.076	1	1	1	2	2	1	1	1	1	1	CAPS	*Alu* I	Present
CT10888	4.078	1	1	1	2	2	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT10184I	4.079	1	1	1	2	2	2	2	2	2	2	SCAR	n/a	Matt Robins, personal comm.
C2_At1g27530	4.088	1	1	1	2	2	1	1	1	1	1	CAPS	*Dpn* II	SGN
TG500	4.098	1	1	1	2	1	1	1	1	1	1	CAPS	*Dde* I	Present
TG500	4.098	1	1	1	2	2	1	1	1	1	1	SCAR	n/a	Present
LEOH10	4.114	1	1	1	2	2	1	1	1	1	1	CAPS	*Bsa* JI	Tomatomap.net

Table 3-1. Continued

Marker	Approximate position[z]	Allele										Marker type	Restriction enzyme	Reference source for primers
		Fla.7776	Fla.7946	PI114490	PI128216	PI126932	H7981	H7998	Fla.8233	Fla.8517	Fla.8326			
C05HBa0261K11	5.007	1	1	1	2	1	1	1	1	1	1	CAPS	*Tsp* 509I	Present
TG441	5.008	1	1	1	2	2	1	1	1	1	1	CAPS	*Taq* I	Ballvora et al, 2001
P11M6	5.015	1	1	1	2	2	1	1	1	1	1	CAPS	*Taq* I	Ballvora et al, 2001
C2_At1g26945	5.018	1	1	1	2	2	1	1	1	1	1	CAPS	*Dpn* II	SGN
Bs4	5.022	1	1	1	2	2	1	1	1	1	1	CAPS	*Dpn* II	SGN
CT93	5.037	1	1	1	2	2	1	1	1	1	1	CAPS	*Alu* I	Present
C2_At1g13380	5.039	1	1	1	2	1	1	1	1	1	1	CAPS	*Hpy* 188I	SGN
CT20210I	5.045	1	1	1	2	2	1	1	2	2	1	SCAR	n/a	Matt Robins, personal comm.
C2_At4g24830	5.051	1	1	1	2	1	1	1	1	1	1	CAPS	*Hpy* CH4IV	SGN
C2_At4g24830	5.051	1	1	1	2	3	1	1	1	1	1	CAPS	*Tsp* 509I	SGN
TOM152	5.058	1	1	1	2	3	4	4	2	2	1	SSR	n/a	Tomatomap.net
C2_At1g14000	5.060	1	1	1	1,2	2	1	1	1	1	1	CAPS	*Spe* I	SGN
TOM49	5.068	1	1	1	2	2	4	4	2	2	1	SSR	n/a	Tomatomap.net
CT10591	5.076	1	1	1	2	1	1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
LEOH316	5.078	1	1	1	2	1	1	2	2	2	1	CAPS	*Mbo* II	Tomatomap.net
Rx3-L1	5.079	1	1	1	2	1	1	1	2	2	1	CAPS	*Bsr* BI	Tomatomap.net
CosOH73	5.082	1	1	1	2	2	1	2	1	2	1	CAPS	*Alu* I	Tomatomap.net
C2_At3g55800	5.090	1	1	1	2	1	1	1	1	1	1	CAPS	*Hinc* II	SGN
TG351	5.094	1	1	1	2	1	1	1	1	1	1	CAPS	*Mwo* I	Present

Table 3-1. Continued

Marker	Approximate position[z]	Allele										Marker type	Restriction enzyme	Reference source for primers
		Fla.7776	Fla.7946	PI114490	PI128216	PI126932	H7981	H7998	Fla.8233	Fla.8517	Fla.8326			
C06HBa0107A05	6.003	1	1	2	2	1	1	1	1	1	1	CAPS	*Mbo* II	Maxwell et al. 2008
C2_At1g07080	6.004	1	1	2	2	2	1	1	1	1	1	CAPS	*Ban* I	SGN
C06HBa0185J10	6.005	1	1	2	2	2	1	1	1	1	1	CAPS	*Bsm* FI	Maxwell et al. 2008
LE_HBa0037I09	6.005	1	1	2	2	2	1	1	1	1	1	CAPS	*Bse* RI	Maxwell et al. 2008
C06HBa0019E05	6.006	1	1	2	2	2	1	1	1	1	1	CAPS	*Apo* I	Maxwell et al. 2008
C8B	6.006	1	1	2	2	2	1	1	1	1	1	CAPS	*Hha* I	Yuanfu Ji, personal comm.
CT119	6.006	1	1	2	2	2	1	1	1	1	1	CAPS	*Rsa* I	SGN
SSR47	6.007	1	1	2	2	3	3	1	1	1	1	SSR	n/a	SGN
CT10242I	6.007	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT10242	6.007	1	1	2	2		1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
TG97	6.009	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	Yuanfu Ji, personal comm.
T1456	6.010	1	1	2	2	2	2	2	1	1	1	CAPS	*Rsa* I	Ji et al. 2007
CT10187	6.011	1	1	2	2	2	2	2	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
C2_At3g10920	6.013	1	1	1	1	1	2	2	1	1	1	CAPS	*Dpn* II	SGN
C2_At3g10920	6.013	1	1	2	2	2	1	1	1	1	1	CAPS	*Alu* I	SGN
C2_At3g56130	6.017	1	1	1	1	2	1	1	1	1	1	CAPS	*Hpy* CH4III	Yuanfu Ji, personal comm.
TG352	6.018	1	1	1	2	1	1	1	1	1	1	CAPS	*Taq* I	Ji et al. 2007
TG590 F2R2	6.022	1	1	1	2	2	1	1	1	1	1	CAPS	*Hpy* CH4III	Maxwell et al. 2008
FLUW25	6.025	1	1	1	2	2	1	1	1	1	1	CAPS	*Taq* I	Maxwell et al. 2008
P6-25F2R5	6.025	1	1	1	2	2	1	1	1	1	1	CAPS	*Taq* I	Yuanfu Ji, personal comm.
CT10328I	6.027	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
T0834-F1a,R2	6.032	1	1	1	2	2	1	1	1	1	1	SCAR	n/a	Maxwell et al. 2008
C2_At1g44760	6.040	1	1	1	1	2	1	3	1	1	1	CAPS	*Alu* I	SGN
C2_At1g44760	6.040	1	1	1	1	1	1	2	1	1	1	CAPS	*Mse* I	SGN
C2_At1g44760	6.040	1	1	1	2	2	1	1	1	1	1	CAPS	*Nsi* I	SGN
TG356	6.044	1	1	1	2	1	1	1	1	1	1	CAPS	*Taq* I	Present
TG435	6.058	1	1	1	2	2	1	1	1	1	1	SCAR	n/a	Yuanfu Ji, personal comm.
SP	6.066	1	1	2	2	2	2	2	1	1	1	CAPS	*Bst* NI	Tomatomap.net
LEOH200	6.069	1	1	1	2	1	1	1	1	1	1	CAPS	*Eco* RV	Tomatomap.net
LEOH112	6.072	1	1	1	2	2	1	1	1	1	1	CAPS	*Hpy* CH4IV	Tomatomap.net
SCBC 792	6.075	1	1	2	3	3	1	1	1	1	1	SNP	Cel I	Yuanfu Ji, personal comm.
cLES-1-K3	6.090	1	1	2	1	1	1	1	1	1	1	CAPS	*Age* I	Yuanfu Ji, personal comm.

Table 3-1. Continued

Marker	Approximate position[z]	Allele										Marker type	Restriction enzyme	Reference source for primers
		Fla.7776	Fla.7946	PI114490	PI128216	PI126932	H7981	H7998	Fla.8233	Fla.8517	Fla.8326			
CT20017	7.003	1	2	1	2	2	1	2	2	1	2	SNP	n/a	Sung-Chur Sim, personal comm.
C2_At5g20180	7.006	1	1	1	2	2	1	1	1	1	1	CAPS	*Bst* UI	SGN
C2_At5g20180	7.006	1	2	2	2	2	2	2	2	2	2	CAPS	*Taq* I	SGN
C2_At2g26590	7.015	1	1	1	2	2				1	1	SNP	Cel I	SGN
C2_At4g29490	7.022	1	1	1	2	2	1	1	1	1	1	CAPS	*Rsa* I	SGN
C2_At1g19140	7.024	1	1	1	2	2	1	1	1	1	1	SCAR	n/a	SGN
FW7	7.024	1	1	1	2	1	1	1	1	1	1	CAPS	*Taq* I	SGN
SSR276	7.037	1	1	1	2	2	1	1	1	1	1	SSR	n/a	SGN
C2_At4g26680	7.038	1	1	1	2	2	1	1	1	1	1	CAPS	*Alu* I	SGN
TG217	7.073	1	1	1	2	2	1	1	1	1	1	CAPS	*Hpy* CH4IV	SGN
C2_At2g20860	7.043	1	1	1	2	2	1	1	1	1	1	CAPS	*Bsa* BI + *Bsa* JI	SGN
C2_At2g20860	7.043	1	2	1	2	2	1	1	1	1	1	CAPS	*Mwo* I	SGN
C2_At2g20860	7.043	1	2	1	1	1	1	1	1	1	1	CAPS	*Nla* III	SGN
C2_At1g53670	7.054	1	1	1	2	1	1	1	1	1	1	CAPS	*Hha* I	SGN
TG216-1	7.062	1	1	2	2	2	1	1	1	1	1	CAPS	*Bsl* I	Aliya Momotaz, personal comm.
TG174	7.065	1	1	1	2	2	1	1	1	1	1	CAPS	*Hha* I	SGN
LEOH40	7.066	1	1	2	1	1	2	2	1	2	1	CAPS	*Tsp* 45I	Yang et al. 2004
LEOH1.1	7.066	1	1	1	1	2	1	2	1	2	1	CAPS	*Tsp* 45I	Tomatomap.net
SSR45	7.080	1	1	2	3	3	4	2	5	1	1	SSR	n/a	SGN
CT20051	7.[y]	1	2	1	2	2	1	2	2	1	2	SNP	n/a	Sung-Chur Sim, personal comm.

Table 3-1. Continued

Marker	Approximate position[z]	Allele										Marker type	Restriction enzyme	Reference source for primers
		Fla.7776	Fla.7946	PI114490	PI128216	PI126932	H7981	H7998	Fla.8233	Fla.8517	Fla.8326			
C2_At5g46630	8.000	1	1	1	2	2	1	1	1	1	1	CAPS	*Hpy* CH4III	SGN
U221657	8.013	1	1	1	2	2	1	1	1	1	1	CAPS	*Rsa* I	SGN
U229378	8.017	1	1	1	1	2	1	1	1	1	1	CAPS	*Bst* UI	SGN
U229378	8.017	1	1	2	1	1	1	1	1	1	1	CAPS	*Hinf* I	SGN
LEOH343	8.018	1	1	2	1	1	2	2	1	2	1	CAPS	*Mnl* I	Tomatomap.net
LEOH147	8.019	1	1	1	2	2	1	1	1	1	1	CAPS	*Tsp* 45I	Tomatomap.net
CosOH64	8.020	1	1	2	2	1	1	1	1	1	1	CAPS	*Rsa* I	Tomatomap.net
C2_At5g27390	8.021	1	1	1	2	2	1	1	1	1	1	CAPS	*Dpn* II	SGN
C2_At2g26830	8.030	1	1	1	2	2	1	1	1	1	1	CAPS	*Hpy* CH4III	SGN
C2_At3g43540	8.041	1	1	1	1	1	2	1	1	1	1	CAPS	*Rsa* I	SGN
C2_At3g43540	8.041	1	1	1	2	1	2	1	1	1	1	CAPS	*Aci* I	SGN
C2_At3g43540	8.041	1	1	1	1	2	1	1	1	1	1	SCAR	n/a	SGN
C2_At5g25630	8.042	1	1	1	2	2	1	1	1	1	1	CAPS	*Hpy* CH4IV	SGN
TG302	8.044	1	1	1	1	1	2	1	1	1	1	CAPS	*Taq* I	SGN
TG302	8.044	1	1	1	2	2	2	1	1	1	1	CAPS	*Alu* I	SGN
CosOH42	8.084	1	1	2	1	1	2	2	1	2	1	CAPS	*Tsp* 45I	Tomatomap.net

Table 3-1. Continued

Marker	Approximate position[z]	Allele										Marker type	Restriction enzyme	Reference source for primers
		Fla.7776	Fla.7946	PI114490	PI128216	PI126932	H7981	H7998	Fla.8233	Fla.8517	Fla.8326			
TG254	9.000	1	1	2	2	2	1	1	1	2	1	SCAR	n/a	SGN
C2_At2g37025	9.015	1	1	1	2	1	1	1	1	1	1	CAPS	*Hpy* CH4IV	SGN
C2_At2g37025	9.015	1	1	1	1	2	2	2	1	1	1	CAPS	*Taq* I	SGN
C2_At2g41680	9.016	1	1	2	2	3	2	2	1	4	1	SNP	Cel I	SGN
C2_At2g32600	9.017	1	1	1	2	1	1	1	1	1	1	CAPS	*Mwo* I	SGN
C2_At3g09920	9.017	1	1	1	2	1	1	1	1	1	1	CAPS	*Dde* I	SGN
C09HBa0203J14.1	9.023	1	1	1	2	2	1	1	1	1	1	CAPS	*Tsp* 509I	Present
C09HBa0203J14.1	9.023	1	1	1	2	1	1	1	1	1	1	CAPS	*Hinf* I	Present
LEOH31.3	9.039	1	1	1	2	1	1	1	1	1	1	CAPS	*Msp* I	Tomatomap.net
SSR383	9.057	1	1	1	2	1	1	1	2	2	2	SSR	n/a	SGN
LEOH144	9.065	1	1	1	2	2	1	1	1	1	1	CAPS	*Fok* I	Tomatomap.net
TOM236	9.086	1	1	2	3	4	1	5	1	1	1	SSR	n/a	Tomatomap.net
SSR333	9.109	1	1	2	1	3	1	1	1	1	1	SSR	n/a	SGN
Cosi52	9.[y]	1	1	1	2	1	2	2	1	1	1	SCAR	n/a	Tomatomap.net

Table 3-1. Continued

Marker	Approximate position[z]	Allele										Marker type	Restriction enzyme	Reference source for primers
		Fla.7776	Fla.7946	PI114490	PI128216	PI126932	H7981	H7998	Fla.8233	Fla.8517	Fla.8326			
C2_At3g21610	10.000	1	1	1	2	2	1	1	1	1	1	CAPS	*Dpn* II	SGN
C2_At5g06430	10.003	1	1	2	1	1	2	2	1	1	1	SCAR	n/a	SGN
C2_At1g53000	10.008	1	1	1	2	2	1	1	1	1	1	CAPS	*Dpn* II	SGN
T0787	10.009	1	1	1	2	2	1	1	1	1	1	CAPS	*HaeIII*	Present
TG303	10.011	1	1	1	2	1	1	1	1	1	1	CAPS	*Dde* I	SGN
TG303	10.011	1	1	1	1	2	1	1	1	1	1	CAPS	*Dpn* II	SGN
CT10670	10.013	1	1	2	2	2	1	1	1	1		SNP	n/a	Sung-Chur Sim, personal comm.
C2_At5g60990	10.014	1	1	2	2	2	1	1	1	1	1	CAPS	*Hinf* I	SGN
CT10105I	10.030	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
SSR318	10.031	1	1	2	2	3	2	4	1	1	1	SSR	n/a	SGN
SSR248	10.035	1	1	2	2	3	2	4	1	1	1	SSR	n/a	SGN
LEVCOH15	10.037	1	1	2	3	4	5	1	1	1	1	SCAR	n/a	Tomatomap.net
CT10419I	10.043	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
C2_At1g67740	10.044	1	1	2	2	2	1	1	1	1	1	CAPS	*Hinf* I	SGN
TG285	10.045	1	1	2	2	2	1	1	1	1	1	CAPS	*Hpy* CH4III	Present
CT10078I	10.046	1	1	2	1,2	1	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT10701	10.046	1	1	2	2	2	1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
C2_At3g58470	10.061	1	1	1	2	2	1	1	1	1	1	CAPS	*Tsp* 509I	SGN
TG233	10.086	1	1	1	2	1	1	1	1	1	1	CAPS	*Hinc* II	SGN
TG403	10.095	1	1	1	1	1	1	2	1	1	1	CAPS	*Dde* I	Present
TG403	10.095	1	1	2	2	2	2	2	2	2	2	CAPS	Cel I	Present
TG63	10.103	1	1	1	2	1	1	1	1	1	1	CAPS	*Hpy* 188I	Present

Table 3-1. Continued

Marker	Approximate position[z]	Allele										Marker type	Restriction enzyme	Reference source for primers
		Fla.7776	Fla.7946	PI114490	PI128216	PI126932	H7981	H7998	Fla.8233	Fla.8517	Fla.8326			
TG523	11.025	1	1	2	2	2	1	1	1	1	1	SNP	Cel I	Yuanfu Ji, personal comm.
T0408-1,2	11.026	1	1	1	2	2	1	1	1	1	1	CAPS	*Mnl* I	Aliya Momotaz, personal comm.
CT20244I	11.034	1	1	2	1	1	1	2	1	2	1	SCAR	n/a	Matt Robins, personal comm.
C2_At4g22260	11.037	1	1	2	1	1	2	2	1	2	1	CAPS	*Dde* I	SGN
CT182	11.038	1	1	2	1	1	2	2	1	2	1	CAPS	*Rsa* I	Yuanfu Ji, personal comm.
cLEX-4-G10	11.044	1	1	2	2	1	1	1	1	1	1	CAPS	*Rsa* I	Yuanfu Ji, personal comm.
CT10737I	11.053	1	2	1	1,2	2	2	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
TG286-3	11.054	1	1	1	1	2	1	2	2	1	2	CAPS	*Hpy* CH4IV	Aliya Momotaz, personal comm.
CosOH57	11.054	1	1	2	2	1	2	2	1	1	1	CAPS	*Pfl* FI	Tomatomap.net
TG400	11.057	1	1	1	1	1	2	1	1	1	1	CAPS	*Mnl* I	SGN
CT10615I	11.059	1	1	1	2	1	2	2	1	1	1	SCAR	n/a	Matt Robins, personal comm.
TG384	11.059	1	1	2	2	1	1	2	1	1	1	CAPS	*Hha* I	Yuanfu Ji, personal comm.
SSR637	11.059	1	2	3	3	2	2	1	1	1	1	SSR	n/a	Tomatomap.net
cTOE-14-L16	11.060	1	1	2	2	3	3	1	1	1	1	SNP	Cel I	Yuanfu Ji, personal comm.
TOM196	11.061	1	2	1	1	3	3	1	1	1	1	SSR	n/a	Tomatomap.net
TOM144	11.062	1	2	3	3	2	4	1	1	1	1	SSR	n/a	Tomatomap.net
CT20181	11.068	1	1	2	2	2	1	2	2	2	2	SNP	n/a	Sung-Chur Sim, personal comm.
C2_At3g54470	11.072	1	1	2	2	2	1	2	2	2	2	SNP	Cel I	SGN
C2_At1g30825	11.072	1	1	2	2	1	1	1	1	2	1	CAPS	*Bse* RI	SGN
cLET-24-J2	11.094	1	1	2	2	1	2	2	1	1	1	CAPS	*Hpy* CH4III	Yuanfu Ji, personal comm.
LEOH57	11.[y]	1	2	2	2	2	2	1	1	1	1	CAPS	*Bst* UI	David Francis, personal comm.

Table 3-1. Continued

Marker	Approximate position[z]	Allele										Marker type	Restriction enzyme	Reference source for primers
		Fla.7776	Fla.7946	PI114490	PI128216	PI126932	H7981	H7998	Fla.8233	Fla.8517	Fla.8326			
CT10925I	12.001	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT10953I	12.029	1	1	2	2	2	1	1	1	2	1	SCAR	n/a	Matt Robins, personal comm.
TG360	12.032	1	1	1	2	2	1	1	1	2	1	CAPS	*Apo* I	Aliya Momotaz, personal comm.
CT100	12.036	1	1	2	2	2	1	1	1	2	1	CAPS	*Rsa* I	Tomatomap.net
SSR20	12.037	1	2	1	1	1	2	1	1	2	2	SSR	n/a	SGN
C2_At4g16710	12.040	1	1	1	2	1	1	1	1	1	1	CAPS	*Taq* I	SGN
CT99	12.045	1	1	1	2	3	1	1	1	2	1	CAPS	*Hpy* CH4III	SGN
TG565	12.048	1	1	1	2	2	1	1	1	1	1	CAPS	*Alu* I	Aliya Momotaz, personal comm.
T1736	12.052	1	1	1	2	2	1	1	1	1	1	SCAR	n/a	SGN
C2_At5g42740	12.055	1	1	1	2	2	1	1	1	2	1	CAPS	*Dde* I	SGN
C2_At4g18593	12.059	1	1	2	1	1	1	1	1	1	1	CAPS	*Sau* 96I	SGN
LEOH301	12.063	1	1	1	2	2	1	1	1	1	1	SCAR	n/a	Tomatomap.net
CosOH1	12.070	1	1	2	2	2	1	1	1	1	1	CAPS	*Tsp* RI	Tomatomap.net
LEOH275	12.071	1	1	1	2	2	1	1	1	1	1	CAPS	*Mse* I	Tomatomap.net
CT10329I	12.078	1	1	2	3	3	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT10778	12.079	1	1	2	2	2	1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
CT10796I	12.080	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
LEOH197	12.081	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	Tomatomap.net
PtiB	12.[y]	1	1	2	2	2	1	1	1	1	1	CAPS	*Mnl* I	Tomatomap.net

Table 3-1. Continued

Marker	Approximate position[z]	Allele										Marker type	Restriction enzyme	Reference source for primers
		Fla.7776	Fla.7946	PI114490	PI128216	PI126932	H7981	H7998	Fla.8233	Fla.8517	Fla.8326			
CT20156I	not mapped	1	1	2	2	1	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT10042I	not mapped	1	1	2	1	1	2	2	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT10100I	not mapped	1	1	1	2	2	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT20074I	not mapped	1	1	1	2	2	1	1	1	1	1	SCAR	n/a	Matt Robins, personal comm.
CT20202I	not mapped	1	1	1	2	2	1	1	2	2	1	SCAR	n/a	Matt Robins, personal comm.
SSR71	not mapped	1	1	2	2	2		3	1	1	1	SCAR	n/a	SGN
C2_At3g54360	not mapped	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	SGN
C2_At2g25950	not mapped	1	1	2	2	2	1	1	1	1	1	SCAR	n/a	SGN
CT10793	not mapped	1	1	2	2	1	1	1	1	2	2	SNP	n/a	Sung-Chur Sim, personal comm.
CT10483	not mapped	1	1		2		1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
CT10218	not mapped	1	1	2	2	2	1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
CT10432	not mapped	1	1	2	2	2	2	2	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
CT10012	not mapped	1	1	1,2	1,2	1	1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
CT10050	not mapped	1	1	2	2	2	1	1	1	2		SNP	n/a	Sung-Chur Sim, personal comm.
CT10943	not mapped	1	1	2	2	2	1	1	1	1		SNP	n/a	Sung-Chur Sim, personal comm.
CT10425	not mapped	1	1	2	2	2	1	1	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.
CT20001	not mapped	1	2	2	2	2	2	1	1	1	2	SNP	n/a	Sung-Chur Sim, personal comm.
CT10205	not mapped	1	1	1	1	1	1	2	1	1	1	SNP	n/a	Sung-Chur Sim, personal comm.

[z] Number represents chromosome and map position (in cM).

[y] Precise map position has not been determined.

Table 3-2. Disease severity on resistant and susceptible selections from the Florida 7776 x Florida 8233 F_2 generation, and subsequent progeny in later seasons.

	Season								
	Fall 2006			Spring 2007			Summer 2007		
Selection	Generation	Plot designation	Disease severity[z]	Generation	Plot designation	Disease severity[z]	Generation	Plot designation	Disease severity[z]
Resistant									
Fla. 8233	Inbred	E501	3.1 ± 0.24	Inbred	E501	3.3 ± 0.45	Inbred	E740	3.5 ± 0.51
1	F_2	E507-281	3.0	F_3	E535	2.3 ± 0.57	F_3	E748	4.3 ± 0.97
2	F_2	E507-364	3.0	F_3	E538	2.7 ± 0.83	F_3	E749	3.1 ± 0.32
3				F_2	E507-31	2.0	F_3	E757	3.0 ± 0.00
4	F_2	E507-225[y]	3.0	F_3	E528-2	2.0	F_4	E742	3.9 ± 0.97
5	F_2	E507-264	3.0	F_3	E532	2.2 ± 0.53	F_3	E746	4.1 ± 1.03
6	F_2	E507-273	3.0	F_3	E534	2.7 ± 0.89	F_3	E747	3.9 ± 0.93
7							F_2	E707-20	3.0
8							F_2	E707-22	3.0
9							F_2	E707-27	3.0
10							F_2	E707-31	2.5
11							F_2	E707-191	3.0
Susceptible									
Fla. 7776	Inbred	E502	4.1 ± 0.47	Inbred	E502	5.6 ± 0.62	Inbred	E741	4.5 ± 0.52
1	F_2	E507-217	5.0	F_3	E527	3.1 ± 0.94	F_3	E861	5.5 ± 0.51
2	F_2	E507-225[y]	3.0	F_3	E528-13	6.0	F_4	E743	5.1 ± 0.56
3							F_2	E707-9	5.5
4							F_2	E707-65	6.0
5							F_2	E707-182	6.0

[z] Horsfall-Barratt (1945) scale. Lower numbers indicate less disease, see text.

[y] E507-225 progeny segregated for resistance, see text.

Table 3-3. Genotypic data on resistant and susceptible progeny selections (see Table 3-2) for markers polymorphic between Florida 8233 and Florida 7776.

						Selection															
		Probable		Parent		Resistant											Susceptible				
Marker	Chromosome	allele source	*P*	Fla.8233	Fla.7776	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5
SSR478	1	undetermined	0.143	+[z]	-[y]	+[x]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
U237757	1	H7998		+	-	-[w]	-	-	+	+	-	-	+	-	+	-	+	+	-	-	+
SSR111	3	undetermined	0.691	+	-	-	-	/[v]	+	+	+	/	-	-	/	-	/	+	-	/	/
CT20145	4	PI 128216		+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CT10184I	4	PI 128216	0.256	+	-	+	-	+	+	+	+	/	/	+	+	+	+	+	+	+	+
CT10184	4	PI 128216	0.169	+	-	+	-	+	+	+	+	/	/	+	/	+	+	+	+	+	+
C2_At5g37360	4	undetermined	0.750	+	-	/	+	/	+	/	/	/	/	-	+	+	+	+	-	+	/
CT20210I	5	PI 128216	0.464	+	-	/	/	/	-	/	+	-	+	-	/	/	+	-	-	/	-
TOM152	5	PI 128216	0.464	+	-	/	/	/	-	/	+	-	+	-	/	/	+	-	-	/	-
TOM49	5	PI 128216	0.339	+	-	+	+	+	-	+	+	-	/	-	/	+	+	-	/	/	-
LEOH316	5	PI 128216	0.267	+	-	-	+	+	+	+	+	+	+	-	/	+	+	-	/	-	+
Rx3-L1	5	PI 128216	0.104	+	-	-	-	/	-	-	-	/	/	-	/	+	-	-	-	-	-
CT20051	7	undetermined	0.836	+	-	-	-	+	/	+	-	-	/	/	/	+	+	-	/	/	+
CT20017	7	undetermined	0.377	+	-	-	-	/	/	+	-	-	/	/	/	+	+	-	/	/	+
C2_At5g20180	7	undetermined	0.222	+	-	-	-	/	/	+	-	/	/	-	/	+	/	+	/	-	+
SSR45	7	undetermined	0.465	+	-	-	/	-	+	+	-	+	+	+	-	+	/	+	-	-	/
SSR383	9	PI 128216	0.108	+	-	-	+	/	-	+	+	/	+	+	/	+	/	-	/	/	-
TG403	10	PI 128216	0.059	+	-	/	+	-	-	/	-	/	/	+	/	/	-	-	-	-	/
C2_At3g54470	11	H7998	0.363	+	-	-	-	/	-	-	-	/	/	-	/	/	-	-	/	-	-
TG286-3	11	H7998	0.104	+	-	-	-	/	-	-	-	/	/	-	+	/	-	-	-	-	-
CT20181	11	H7998	0.104	+	-	-	-	/	-	-	-	/	/	-	+	/	-	-	-	-	-

[z] Homozygous for the Fla. 8233 allele.

[y] Homozygous for the Fla. 7776 allele.

[x] SSR478 is a dominant marker: "+" here represents genotypes that are either homozygous or heterozygous for the Fla. 8233 allele.

[w] U237757 is a dominant marker: "-" here represents genotypes that are either homozygous or heterozygous for the Fla. 7776 allele.

[v] Heterozygous.

Table 3-4. Disease severity on resistant and susceptible selections from the Florida 7776 x Florida 8517 F_2 generation, and subsequent progeny in later seasons.

	Season								
	Fall 2006			Spring 2007			Summer 2007		
Selection	Generation	Plot designation	Disease severity[z]	Generation	Plot designation	Disease severity[z]	Generation	Plot designation	Disease severity[z]
Resistant									
Fla. 8517	Inbred	E508	3.2 ± 0.22	Inbred	E508	1.6 ± 0.16	Inbred	E761	3.3 ± 0.47
1	F_2	E514-246[y]	3.0	F_3	E548-7	2.0	F_4	E763	3.9 ± 0.74
2	F_2	E514-278	3.0	F_3	E551	2.8 ± 0.86	F_3	E770	4.1 ± 0.64
3	F_2	E514-395	3.8	F_3	E562	4.7 ± 1.02	F_3	E773	3.6 ± 0.61
4	F_2	E514-385	3.5				F_3	E776	3.8 ± 0.87
5							F_2	E714-27	3.0
6							F_2	E714-152	3.0
7							F_2	E714-153	2.5
8							F_2	E714-164	3.5
9							F_2	E714-191	4.5
Susceptible									
Fla. 7776	Inbred	E509	4.2 ± 0.45	Inbred	E502	5.6 ± 0.62	Inbred	E762	5.4 ± 0.50
1	F_2	E514-277	4.5	F_3	E550	4.0 ± 0.94	F_3	E862	5.9 ± 0.35
2	F_2	E514-380	4.0	F_3	E561-9	2.0	F_4	E864	5.5 ± 0.52
3	F_2	E514-246[y]	3.0	F_3	E548-3	6.0	F_4	E764	5.3 ± 0.48
4	F_2	E514-238	4.0	F_3	E546	2.6 ± 0.61	F_3	E768	5.2 ± 0.92
5							F_2	E714-32	6.0
6							F_2	E714-88	5.5
7							F_2	E714-156	5.5
8							F_2	E714-188	5.3

[z] Horsfall-Barratt (1945) scale. Lower numbers indicate less disease, see text.

[y] E514-246 progeny segregated for resistance, see text.

Table 3-5. Genotypic data on resistant and susceptible progeny selections (see Table 3-4) for markers polymorphic between Florida 8517 and Florida 7776.

						Selection															
		Probable		Parent		Resistant									Susceptible						
Marker	Chromosome	allele source	*P*	Fla.8517	Fla.7776	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8
SSR478	1	undeterminable	0.259	+[z]	-[y]	+[x]	+	+	-	+	-	+	+	+	+	+	-	-	-	-	+	+
SSR96	2	PI 114490	0.488	+	-	-	-	-	-	/[w]	/	/	/	/	-	-	-	-	/	-	/	/
SSR104	2	PI 114490	0.257	+	-	-	-	-	-	+	/	/	+	/	-	-	-	-	/	-	/	/
LEOH23.3	2	PI 114490	0.565	+	-	-	-	-	-	+	+	/	+	/	-	-	-	-	+	-	/	+
CT10050	not mapped	PI 114490	0.329	+	-	-	-	-	-	+	+	+	+	+	-	-	/	-	+	-	-	+
CT10649	2	PI 114490	0.177	+	-	+	+	+	+	+	/	/	+	/	/	+	+	+	/	/	-	/
SSR5	2	PI 114490	0.177	+	-	+	+	+	+	+	/	/	+	/	/	+	+	+	/	/	-	/
SSR349A	2	PI 114490	0.177	+	-	+	+	+	+	+	/	/	+	/	/	+	+	+	/	/	-	/
TOM188	2	PI 114490	0.177	+	-	+	+	+	+	+	/	/	+	/	/	+	+	+	/	/	-	/
CT10793	not mapped	PI 114490	0.177	+	-	+	+	+	+	+	/	/	+	/	/	+	+	+	/	/	-	/
CT10923	2	PI 114490	0.277	+	-	+	/	/	+	+	/	/	+	/	/	/	+	+	/	/	-	/
CT10153	2	PI 114490	0.826	+	-	+	/	/	+	+	/	/	+	/	+	+	+	+	/	/	-	/
CT10771	2	PI 114490	0.609	+	-	+	+	+	+	+	+	/	+	/	+	+	+	+	+	+	-	/
CT20037	3	PI 114490	0.005	+	-	-	-	-	/	/	/	/	+	/	-	-	-	-	-	-	-	-
CT10736	3	PI 114490	0.005	+	-	-	-	-	/	/	/	/	+	/	-	-	-	-	-	-	-	-
C2_At1g02140	3	PI 114490	0.005	+	-	-	-	-	/	/	/	/	+	/	-	-	-	-	-	-	-	-
C2_At5g62390	3	PI 114490	0.005	+	-	-	-	-	/	/	/	/	+	/	-	-	-	-	-	-	-	-
CT20145	4	Fla.7600	0.304	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	/	+	+
CT10184I	4	Fla.7600	0.521	+	-	+	+	+	+	/	+	/	+	+	+	+	+	/	+	/	/	+
CT10184	4	Fla.7600	0.259	+	-	+	+	+	+	/	+	/	+	+	+	+	+	/	/	/	/	+
C2_At5g37360	4	Fla.7600	0.935	+	-	/	+	/	/	+	-	/	+	/	+	/	-	/	/	+	+	/
CT20210I	5	OH9242	0.596	+	-	-	/	+	-	-	-	-	-	-	/	/	-	/	/	-	-	-
TOM152	5	OH9242	0.596	+	-	-	/	+	-	-	-	-	-	-	/	/	-	/	/	-	-	-
TOM49	5	OH9242	0.312	+	-	+	/	+	+	-	-	-	-	-	/	-	-	/	/	-	-	-
LEOH316	5	OH9242	0.227	+	-	/	/	+	-	+	-	-	/	+	-	-	-	/	+	-	/	-
Rx3-L1	5	OH9242	0.620	+	-	/	/	-	+	-	-	-	-	-	/	+	-	/	-	/	-	-
CosOH73	5	PI 128216	0.230	+	-	/	/	+	+	+	-	-	/	+	/	+	-	+	/	/	-	-

Table 3-5. Continued

Marker	Chromosome	Possible allele source	*P*	Parent		Selection															
						Resistant									Susceptible						
				Fla.8517	Fla.7776	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8
C2_At5g20180	7	undetermined	0.792	+	-	/	/	/	-	-	/	/	+	/	-	-	+	+	+	+	-	-
LEOH1.1	7	OH9242		+	-	+	-[v]	-	-	-	+	-	+	-	-	+	-	+	-	+	-	-
LEOH40	7	OH9242	0.809	+	-	+	/	-	-	-	+	-	+	/	-	+	/	+	-	+	-	/
LEOH343	8	OH9242	0.812	+	-	-	-	-	-	-	-	/	+	-	-	-	-	-	-	-	+	-
CosOH42	8	undetermined		+	-	+	-[v]	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-
TG254	9	undetermined		+	-	-[v]	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
C2_At2g41680	9	undetermined	0.488	+	-	-	+	/	/	+	-	/	+	/	+	/	-	+	/	+	+	/
SSR383	9	PI 128216	0.157	+	-	-	/	+	+	/	/	+	-	/	/	/	/	-	-	-	/	/
TG403	10	PI 114490/PI 128216	0.488	+	-	+	-	/	-	+	+	-	+	/	/	+	+	+	/	/	/	/
CT20244I	11	PI 114490/H7998	1.000	+	-	-	+	/	-		+	+	/	-	-	+	-	+		+	/	-
C2_At4g22260	11	PI 114490/H7998	0.566	+	-	-	+	/	-	/	+	+	/	-	-	+	-	+	-	/	/	-
CT182	11	PI 114490/H7998	0.179	+	-	-	+	/	-	/	+	+	/	-	-	+	-	+	-	/	/	-
C2_At3g54470	11	PI 128216	0.047	+	-	-	-	+	-	/	/	-	-	+	-	-	-	-	-	-	-	-
C2_At1g30825	11	PI 128216	0.019	+	-	-	-	+	-	/	/	/	-	+	-	-	-	-	-	-	-	-
CT20181	11	PI 128216	0.018	+	-	-	-	/	-	/	/	/	-	+	-	-	-	-	-	-	-	-
CT100	12	PI 128216		+	-	-[v]	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-
TG360	12	PI 128216	0.585	+	-	-	-	-	-	+	/	/	-	-	-	-	-	-	-	-	+	-
CT99	12	PI 128216	0.585	+	-	-	-	-	-	+	/	/	-	-	-	-	-	-	-	-	+	-
CT10953I	12	PI 128216	0.682	+	-	-	-	-	-	+	/	-	-	+	-	-	-	-	+	-	+	+
C2_At5g42740	12	PI 128216	0.327	+	-	+	/	+	+	+	-	-	+	+	/	+	-	+	+	/	-	-
SSR20	12	Fla.7600/OH9242	0.009	+	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	/	-	+

[z] Homozygous for the Fla. 8517 allele.

[y] Homozygous for the Fla. 7776 allele.

[x] SSR478 is a dominant marker: "+" here represents genotypes that are either homozygous or heterozygous for the Fla. 8517 allele.

[w] Heterozygous.

[v] LEOH1.1, CosOH42 , TG254 and CT100 are a dominant markers: "-" here represents genotypes that are either homozygous or heterozygous for the Fla. 7776 allele.

Table 3-6. Disease severity on resistant and susceptible selections from the Florida 7946 x Florida 8326 F_2 generation, and subsequent progeny in later seasons.

	Season											
	Spring 2006			Spring 2007			Summer 2007			Spring 2008		
Selection	Generation	Plot designation	Disease severity[z]	Generation	Plot designation	Disease severity[z]	Generation	Plot designation	Disease severity[z]	Generation	Plot designation	Disease severity[z]
Resistant												
Fla. 8326	Inbred	E701	3.6 ± 0.40				Inbred	E730	4.2 ± 0.98	Inbred	E724	2.7 ± 0.36
1	F_2	E707-130	3.3	F_3	E520	3.4 ± 0.76	F_3	E736	4.0 ± 0.69			
2	F_2	E707-141	3.0	F_3	E521	3.0 ± 0.69	F_3	E737	3.9 ± 0.93			
3	F_2	E707-161	3.0	F_3	E523	3.8 ± 1.03	F_3	E738	4.3 ± 0.58			
4	F_2	E707-51	3.0	F_3	E515-13	n/d	F_4	E860	4.4 ± 0.70			
5							F_2	E721-12	4.0	F_3	E725	2.4 ± 0.46
6							F_2	E721-111	3.5	F_3	E728	2.8 ± 0.61
7	F_2	E707-166[y]	3.0	F_3	E524-13	2.0	F_4	E732	4.9 ± 0.83	F_5	E732	0..61
Susceptible												
Fla. 7946	Inbred	E702	7.7 ± 0.58				Inbred	E731	6.8 ± 0.40	Inbred	E722	5.7 ± 0.43
1	F_2	E707-166[y]	3.0	F_3	E524-18	6.0	F_4	E733	6.5 ± 0.51	F_5	E733	5.9 ± 0.49
2	F_2	E707-107	7.0	F_3	E519	5.1 ± 1.08	F_3	E735	6.5 ± 0.51			
3	F_2	E707-170	6.8	F_3	E525	5.2 ± 0.81	F_3	E739	6.2 ± 0.38			
4	F_2	E707-83	7.0	F_3	E516	3.0 ± 0.69	F_3	E734	5.2 ± 0.88	F_5	E731	3.6 ± 0.88
5							F_2	E721-36	7.5	F_3	E726	4.7 ± 0.61
6							F_2	E721-93	7.3	F_3	E727	4.7 ± 1.09
7							F_2	E721-170	7.5	F_3	E729	5.6 ± 0.47
8							F_2	E721-174	7.5	F_3	E730	5.3 ± 0.95

[z] Horsfall-Barratt (1945) scale. Lower numbers indicate less disease.

[y] E707-166 progeny segregated for resistance, see text.

Table 3-7. Genotypic data on resistant and susceptible progeny selections (see Table 3-6) for markers polymorphic between Florida 8326 and Florida 7946.

						Selection														
		Probable		Parent		Resistant							Susceptible							
Marker	Chromosome	allele source	*P*	Fla.8326	Fla.7946	1	2	3	4	5	6	7	1	2	3	4	5	6	7	8
Cf9	1	PI 126932	0.063	+[z]	-[y]	+[x]	+	+	-	+	+	+	-	-	+	+	-	-	-	+
C2_At1g02560	1	PI 126932	0.345	+	-	+	-	+	+	/[w]	+	/	+	+	+	+	+	+	/	/
CT10649	2	undetermined	0.635	+	-	+	+	/	/	+	/	-	+	+	+	+	+	+	-	-
CT10793	not mapped	undetermined	0.876	+	-	+	+	/	+	+	/	-	+	+	+	+	+	+	-	-
SSR111	3	undetermined	0.044	+	-	+	+	+	+	+	+	+	+	+	-	/	/	-	+	+
CT20145	4	PI 126932	0.726	+	-	+	+	+	+	+	+	/	+	+	+	+	+	+	-	+
CT10184I	4	PI 126932	0.877	+	-	+	+	+	/	/	/	+	+	+	+	+	+	/	-	+
CT10184	4	PI 126932	0.877	+	-	+	+	+	/	/	/	+	+	+	+	+	+	/	-	+
C2_At2g20860	7	undetermined	0.317	+	-	/	+	+	/	/	+	+		/	/	/	/	+	/	+
SSR383	9	undetermined	0.330	+	-	+	-	+	+	+	-	-	-	-	-	-	/	+	+	-
TG403	10	PI 126932	0.147	+	-	+	+	+	+	/	+	+	+	/	+	/	+	/	+	-
TOM196	11	H7998	<0.0001	+	-	/	/	+	+	+	+	/		-	-	/	-	-	-	-
SSR637	11	H7998	<0.0001	+	-	+	+	+	+	+	+	/	-	-	-	/	-	-	/	-
TOM144	11	H7998	<0.0001	+	-	+	+	+	+	+	+	/	-	-	-	/	-	-	/	-
LEOH57	11	H7998	<0.0001	+	-	+	+	+	+	+	+	/	-	/	-	/	-	-	/	-
CT10737I	11	H7998	0.0002	+	-	+	/	+	+	+	+	/	-	/	-	/	-	-	/	-
C2_At3g54470	11	H7998	0.016	+	-	+	/	/	+	/	+	-	-	/	-	/	-	-	/	-
TG286-3	11	H7998	0.003	+	-	+	/	/	/	/	+	-	-	/	-	-	-	-	-	-
CT20181	11	H7998	0.003	+	-	+	/	/	/	/	+	-	-	/	-	-	-	-	-	-

[z] Homozygous for the Fla. 8326 allele.

[y] Homozygous for the Fla. 7946 allele.

[x] Cf9 is a dominant marker: "+" here represents genotypes that are either homozygous or heterozygous for the Fla. 8326 allele.

CHAPTER 4

GENETIC CONTROL OF RACE T3 HYPERSENSITIVITY FROM PI 126932 AND THE RELATIONSHIP BETWEEN T3 HYPERSENSITIVITY AND RACE T4 RESISTANCE

Introduction

Bacterial spot of tomato (*Solanum lycopersicum* L.) is one of the most serious diseases that faces Florida tomato production. Four races of the bacterial spot pathogen in tomato have been isolated under field conditions (Jones et al., 2005), and one strain of a fifth race has been identified (Minsavage et al., 2003; Jones, unpublished). In Florida, *Xanthomonas euvesicatoria* race T1 was prevalent until 1991 when *X. perforans* race T3 emerged (Jones et al., 1995). Race T3 was antagonistic to race T1 (Jones et al., 1998) and largely replaced the latter race. *X. perforans* race T4 recently emerged and has been isolated in several locations in Florida since 1998 (Minsavage et al., 2003). Clearly, resistance to races T3 and T4 is needed in Florida. One of the most resistant sources of race T3 resistance is Hawaii 7981 (H7981) (Scott et al., 1995). This resistance is based largely on hypersensitivity (HR) conferred by the incompletely dominant gene *Xv3*, but field resistance is also based on other genes (Scott et al., 2001). Race T3 HR and field resistance have also been identified in PI 128216 and PI 126932 (Scott et al., 1995).

Advanced breeding line Fla. 8326 is a large-fruited, fresh market tomato with resistance to bacterial spot race T3 (Scott, unpublished) and tolerance to other races (Scott, et al., 2006). Resistance sources in its pedigree include PI 126932 and Hawaii 7998 (H7998). T3 resistance is provided, at least in part, by HR from PI 126932, and partial resistance to race T4 is derived from H7998 and possibly from PI 126932 (see Ch. 3). Race T4 overcame the *Xv3*-based hypersensitive resistance in H7981, as well as the HR in PI 128216 and PI 126932 (Jones, unpublished). It was not surprising that race T4 overcame this resistance in all three sources, since the HR in each of these three lines is based upon recognition of a common avirulence gene,

avrXv3 (Astua-Monge, et al., 2000b), and race 4 came about by a mutations in the *avrXv3* gene (Minsavage et al., 2003). It is not known, however, whether the HR in PI 126932 and PI 128216 is conferred by *Xv3* or by a different gene operating by the same mechanism, nor how this trait is inherited in the two PIs.

Advanced breeding line Fla. 8517 is a plum tomato with resistance to race T4 (Scott et al., 2006) from PI 114490 and PI 128216 (see Ch. 3) and HR to race T3 from PI 128216. Florida 8233 is a large-fruited fresh market tomato with resistance to races T3 (Scott, unpublished) and T4 (Scott et al., 2006), and it is race T3 hypersensitive. Race T4 resistance is derived from H7998 and PI 128216, and race T3 HR comes from PI 128216 (see Ch. 3). While multiple genes appear to confer T4 resistance in Fla. 8517 and Fla. 8233, it is not known whether any of these genes are involved in T3 HR from PI 128216.

The objectives of this research were to: 1) determine the genetic control of HR from PI 126932, 2) determine the allelism of the T3 HR genes in H7981, PI 126932, and PI 128216, and 3) test for an association between the T3 hypersensitivity in Fla. 8326, Fla. 8233 and Fla. 8517 and T4 field resistance in each of these lines.

Materials and Methods

Inoculum Preparation, Plant Inoculations and Disease Evaluations

X. perforans races T3 and T4 inoculum were each produced by growing the bacteria on Difco nutrient agar (Becton Dickinson and Company, Sparks, Md.) for 24-36h at 28˚C. Bacterial cells were removed from the agar plates and suspended in either sterile tap water (for fall 2005 experiments) or in 10 mM $MgSO_4 \cdot 7\ H_2O$ (for fall 2006 experiments), and the suspensions were standardized to A_{600}=0.30 (a concentration of approximately 2 to 5 x 10^8 colony forming units (cfu)/mL). For race T3, leaves were infiltrated with the bacterial suspension as described by

Hibberd et al. (1987). Infiltrated plants were subsequently moved to a growth room that was kept at a constant temperature of 24°C with a 16-h light period. Assessments for HR were carried out at 12 to 24 hour intervals for 24 to 72 hours after infiltration. Infiltrated areas exhibiting confluent necrosis within 36 hours were scored as hypersensitive. For race T4 field inoculations, the bacterial suspension was applied by misting the foliage with a backpack sprayer early in the morning before sunrise. Field plants were rated for disease severity in the field using the Horsfall and Barratt scale (1945), where 1 = 0%, 2 = 0%-3%, 3 = 3%-6%, 4 = 6%-12%, 5 = 12%-25%, 6 = 25%-50%, 7 = 50%-75%, 8 = 75%-87%, 9 = 87%-94%, 10 = 94%-97%, 11 = 97%-100%, and 12 = 100% diseased tissue.

Plant Materials

Florida 8326 (T3 hypersensitivity from PI 126932) was crossed to Fla. 8021 and Fla. 7946, both susceptible breeding lines to races T3 and T4 of *X. perforans*; to Fla. 8000, a breeding line with T3 HR from H7981; and to Fla. 8233 (T3 HR from PI 128216). The F_1s were subsequently self-pollinated to produce F_2 seed. In fall 2005, F_1 and F_2 generations of crosses involving Fla. 8021 and Fla. 8000, parents of those crosses, and H7981 were grown in the greenhouse at temperatures ranging from 25 to 35°C. After 4 to 5 weeks, the main stem was removed above the fully expanded third true leaf. Approximately 3 days after topping, plants were inoculated with race T3 for testing for an HR.

Also in fall 2005, parents, F_1, and F_2 generations of the crosses involving Fla. 7946 and Fla. 8233 were included as part of a field experiment in Citra, FL. Seed were sown on 29 July, and plants were transplanted to the field on 9 September. Plants were inoculated with race T4 inoculum on 16 September and rated for disease severity on 19 October. Cuttings were taken from field plants on 22 November and labeled according to family and plant number, rooted

under misters, and grown in the greenhouse at temperatures ranging from 25 to 35°C. After 4 to 5 weeks, plants were topped and tested for T3 HR as described above. Thus, each plant was rated in the field for race T4 disease severity and in the greenhouse for race T3 HR.

Florida 8233 and Fla. 8517 were each crossed to Fla. 7776, a susceptible breeding line to races T3 and T4 of *X. perforans*, and each F_1 was subsequently self-pollinated to produce F_2 seed. Parents, F_1, and F_2 generations of these crosses were included as part of field experiments in Citra and Balm, FL in fall 2006. Seed were sown on 17 July and transplanted to the field in Balm, FL on 25 August, and to the field in Citra, FL on 30 August. Race T4 inoculum was applied on 19 and 20 September to the plants in Citra and Balm, respectively. Plants at Citra were rated for disease severity on 11, 12 October, and plants at Balm were rated on 17, 18 October. Cuttings were taken from plants at both locations the week of 1 October and labeled according to family and plant number, rooted under misters, and grown in the greenhouse at temperatures ranging from 25 to 35°C. After 4 to 5 weeks, plants were topped and tested for T3 HR as described above. Thus, each plant was rated in the field for race T4 disease severity and in the greenhouse for race T3 HR.

For all field experiments, seed were sown in growth rooms in Black Beauty spent coal (Reed Minerals Div., Highland, IN) and transplanted approximately 7 to 10 days later to Speedling® trays (3.8 cm^3 cell size) (Speedling, Sun City, FL) in the greenhouse, where seedlings were grown for four weeks. Plants were then transplanted to field beds that were 20 cm high and 81 cm wide that had been fumigated with 67% methyl bromide : 33% chloropicrin at 197 kg ha^{-1} (175 lbs per acre) and covered with reflective plastic mulch. Plants were spaced 46 cm apart in rows, with 152 cm between rows, staked and tied, and irrigated by drip tape beneath the plastic mulch of each bed. A recommended fertilizer program was followed, and plants were

sprayed with pesticides (excluding copper) as needed throughout the season (Olsen et al., 2007-2008).

Results

Inheritance of the gene for HR to *X. perforans* race T3 was studied in the cross between Fla. 8326 (resistant parent) and Fla. 8021 (susceptible parent). Their F_1 displayed a resistant phenotype (Table 4-1), and the corresponding F_2 segregation fit a 3:1 ratio into resistant and susceptible individuals, indicating HR was conferred by a single, dominant gene. In the cross between Fla. 8326 and Fla. 8000, the F_1 displayed the expected HR phenotype, and the F_2 segregation of resistant and susceptible plants fit a 15:1 ratio (Table 4-1), indicating HR was conferred by two dominant genes. In the cross between Fla. 8233 and Fla. 8326, the F_2 did not segregate any susceptible individuals (Table 4-2), indicating they both have the same gene conferring HR. Thus, results suggest that PI 126932 and PI 128216 share a common T3 HR gene, but that this gene is at a different locus than the T3 HR gene in H7981.

In fall 2005, race T4 disease severity was rated in the field, and cuttings from parents and 41 (Fla. 7946 x Fla. 8326) F_2 plants and were tested for race T3 HR (Table 4-2). Disease severity ratings for race T4 in fall 2006 were very difficult to assess due to extremely poor field conditions at Citra, FL and low disease pressure at Citra and Balm, FL. In spite of this, race T4 field disease severity was rated, and race T3 HR was measured on cuttings of parents, 312 (Fla. 7776 x Fla. 8233) F_2 plants and 289 (Fla. 7776 x Fla. 8517) F_2 plants (Table 4-2). Within each of the three crosses, Chi-square contingency tests were used to evaluate the segregation of F_2 plants for race T4 field resistance and race T3 hypersensitivity to test for a relationship between the two traits. In each F_2 family, the two traits segregated idependently, indicating that there is no relationship between race T3 HR and race T4 field resistance (Table 4-3).

Discussion

In breeding for *X. perforans* race T3 resistance, several sources of resistance are available, some of which are race T3 HR and include H7981, PI 126932 and PI 128216 (Jones et al., 1995). The highest level of resistance in the field was identified in H7981 (Scott et al., 1995), and breeding efforts for race T3 resistance thus focused primarily on H7981 while inheritance of resistance from PI 126932 and PI 128216 was never studied. Interest in the two PIs as resistance donors has increased since the emergence of race T4, which overcame the HR of all three resistance sources but not the field resistance of PI 128216 (Scott et al., 2006); additionally, race T4 resistant breeding lines with resistance presumably derived from these PIs have been developed, providing supportive evidence for the presence of non-HR resistance genes in these backgrounds. Herein we present evidence that genetic control of T3 HR in PI 128216 and PI 126932 is provided by a different locus than in H7981.

It is generally believed that hypersensitive responses are controlled by single dominant genes, but this is not always the case. *X. euvesicatoria* race T1 HR in H7998 appears to be controlled by three factors (Wang et al., 1994; Yu et al., 1995), and T3 HR in H7981 is controlled by a single incompletely dominant gene (Scott et al., 1996). Understanding the genetic control of HR from PI 126932 is important in determining whether this resistance would be needed in one or both parents to obtain a full level of hypersensitive resistance in a hybrid. Because the T3 HR gene in PI 126932 appears to be controlled by a single dominant gene, use of this gene is probably more suitable for hybrid development than *Xv3* from H7981. However, control plants heterozygous for *Xv3* from H7981 were not included in the study; it is therefore not clear whether the T3 HR gene in PI 126932 is truly dominant, or if the screen was not precise enough to distinguish a rapid HR from an intermediate response.

One approach to obtain resistance to multiple races of bacterial spot involves the pyramiding of major resistance genes to each race. Hawaii 7981, PI 126932 and PI 128216 all produce an HR in response to race T3, and all have race T3 field resistance (Scott et al., 1995; Scott et al., 2001). Race T4 overcame the HR of each line (Astua-Monge et al., 2000b), as well as the field resistance of H7981 (Scott, unpublished) and PI 126932 (Scott et al., 2006). Thus, the race T3 hypersensitive genes appear to be the primary contributor to race T3 field resistance in H7981 and PI 126932, and this may be the case in PI 128216 as well. In developing advanced breeding lines with race T3 field resistance, it may be desirable to make crosses among lines that have resistance derived from one or another of these sources. Because PI 126932 and PI 128216 appear to share a common race T3 HR locus, crosses between lines with PI-derived HR would be expected to maintain this trait in all progeny. However, because the PI race T3 HR locus is different from the H7981 *Xv3* locus, selected progeny of crosses between lines with Hawaiian-derived HR and lines with PI-derived HR would need to be screened to be certain that race T3 hypersensitivity was maintained.

The race T3 HR genes in H7981, PI 126932 and PI 128216 all act by recognizing the same avirulence factor, *avrXv3* (Astua-Monge et al., 2000b). Race T4 appears to be associated with mutagenesis of the *avrXv3* gene in tomato race 3 strains (Minsavage et al., 2003). Considering this, the lack of a relationship between race T3 HR and race T4 field resistance is not surprising. The hypothesis that such a relationship might have existed is based on the ideas that either: 1) the race T3 HR gene could contribute a level of non-hypersensitive resistance which, in combination with other resistance genes, is effective against race T4, or 2) the race T3 HR gene is linked to another race T4 resistance QTL, as disease resistance genes often cluster in

the genome (Michelmore and Meyers, 1988). Neither appears to be the case, and race T4 resistance appears independent of race T3 HR.

Table 4-1. Hypersensitivity as measured by time to confluent necrosis in tomato plants infiltrated with *Xanthomonas perforans* race T3 in 2005.

		Frequency distribution of time to confluent necrosis[z]							
	Total plants	Hypersensitive		Non-hypersensitive			Expected		
Pedigree	(no.)	24 hr.	36 hr.	48 hr.	60 hr.	72 hr.	ratio	X^2	*P*
H7981	12	12							
Fla.8326[y]	12	12							
Fla.8021[x]	12			6	5	1			
Fla.8000[w]	12	12							
(Fla.8326 x Fla.8021) F_1	12	10	2						
(Fla.8326 x Fla.8021) F_2	97	46	26	17	7	1	3:1	0.055	0.81
(Fla.8326 x Fla.8000) F_1	12	12							
(Fla.8000 x Fla.8326) F_2	205	184	6	9	3	3	15:1	0.295	0.59

[z] Plants were infiltrated with approximately 2 to 5 x 10^8 cfu (colony forming units) / ml

[y] Race T3 hypersensitivity from PI 126932.

[x] Non-hypersensitive to race T3.

[w] Race T3 hypersensitivity from H7981.

Table 4-2. Hypersensitivity to race T3 of *Xanthomonas perforans* on rooted tomato cuttings in fall 2005 and fall 2006.

Season	Pedigree	Total no. of plants	No. of plants reaching confluent necrosis over time[z]				
			24 hr.	36 hr.	48 hr.	60 hr.	72 hr.
2005							
	Bonny Best	1					1
	Fla.7946	1				1	
	Fla.8326	2	2				
	Fla.8233	3	3				
	(Fla.7946 x Fla.8326) F_1	3		1	2		
	(Fla.8326 x Fla.7946) F_1	2	2				
	(Fla.8233 x Fla.8326) F_1	2	2				
	(Fla.8233 x Fla.8326) F_2	102	102				
	(Fla.7946 x Fla.8326) F_2	41	18	7	12	3	1
2006							
	Fla.8233	7	7				
	(Fla.8233 x Fla.7776) F_1	9			8		1
	(Fla.7776 x Fla.8233) F_1	9	1		8		
	(Fla.7776 x Fla.8233) F_2	312	43		175		91
	Fla.7776	15					15
	(Fla.8517 x Fla.7776) F_1	10	4		6		
	(Fla.7776 x Fla.8517) F_1	9	4		5		
	(Fla.7776 x Fla.8517) F_2	289	61		94		134
	Fla.8517	3	3				

[z] Plants were infiltrated with approximately 2 to 5 x 10^8 cfu (colony forming units) / ml

Table 4-3. Segregation of plants for bacterial spot race T3 hypersensitivity and race T4 field resistance in three F_2 families.

			T3 hypersensitivity[z]			
Pedigree	Year	T4 field resistance	HR^+	HR^-	Contingency X^2	*P*
(Fla.7946 x Fla.8326) F_2	2005	Resistant[y]	8	0	1.507	0.220
		Susceptible[x]	15	3		
(Fla.7776 x Fla.8233) F_2	2006	Resistant[w]	31	12	1.430	0.232
		Susceptible[v]	12	9		
(Fla.7776 x Fla.8517) F_2	2006	Resistant[w]	40	23	0.017	0.895
		Susceptible[v]	18	11		

[z] Upon infiltration with approximately 2 to 5 x 10^8 cfu (colony forming units) / ml, plants were considered HR^+ if they exhibited confluent necrosis by 48 hours, and HR^- if the reaction occurred after 48 hours.

[y] Plants were considered resistant if they were rated 4.5 or lower on the Horsfall-Barratt (1945) scale.

[x] Plants were considered susceptible if they were rated 5.5 or higher on the Horsfall-Barratt (1945) scale.

[w] Plants were considered resistant if they were rated 3 or lower on the Horsfall-Barratt (1945) scale.

[v] Plants were considered susceptible if they were rated 4.5 or higher on the Horsfall-Barratt (1945) scale.

APPENDIX A
PEDIGREES

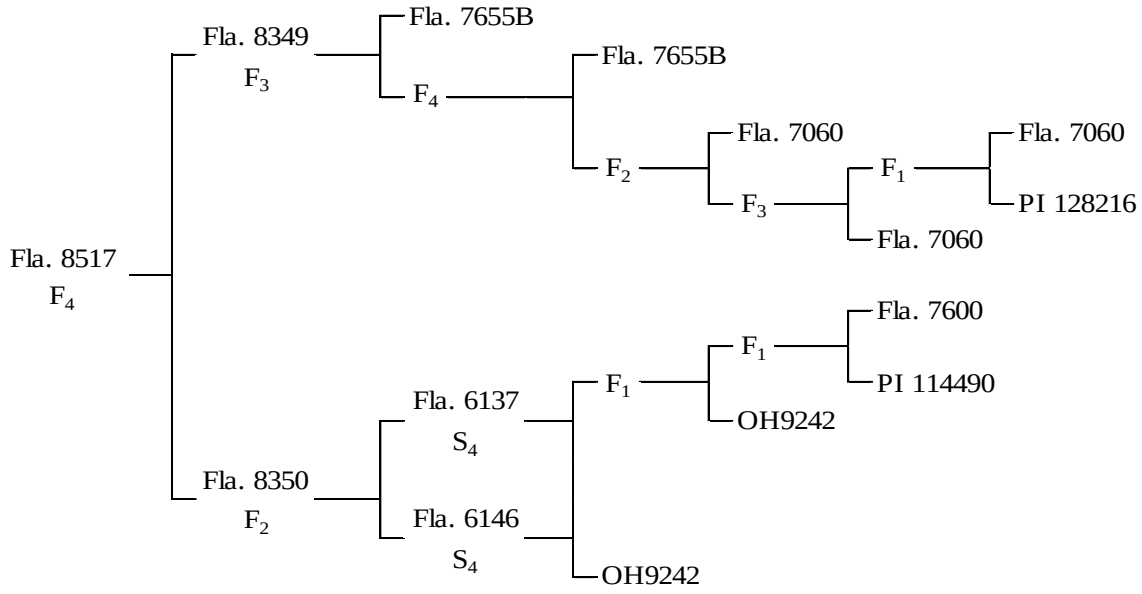


Figure A-1. Pedigree of Fla. 8517. (Both Fla. 7655B and Fla. 7600 contain H7998 in their pedigrees.)

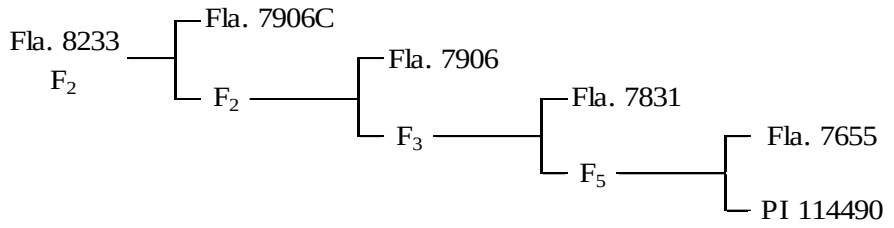


Figure A-2. Putative pedigree of Fla. 8233. [However, PI 114490 is now thought to be incorrectly recorded in this pedigree. It appears that PI 128216 was actually crossed to Fla. 7655 (see Ch. 3).] (Fla. 7655 contains H7998 in its pedigree.)

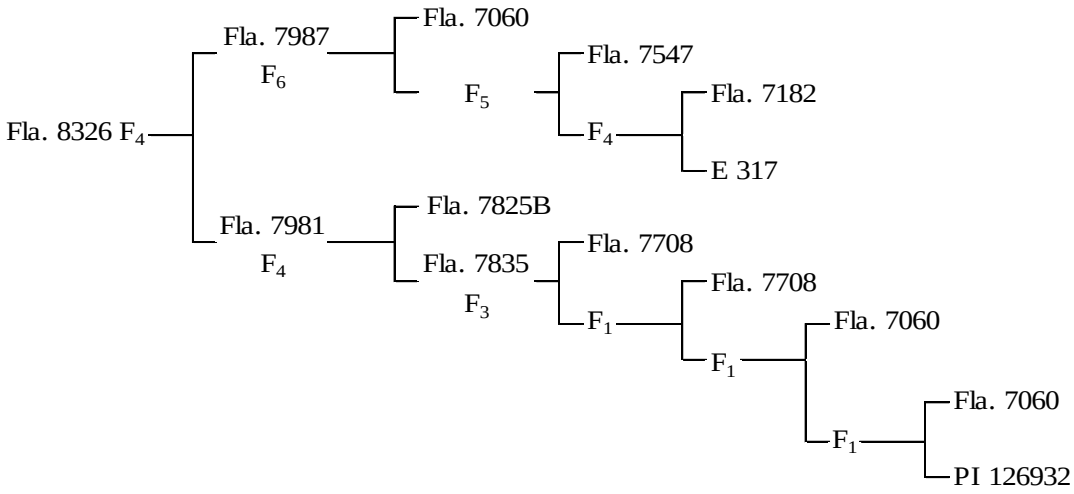


Figure A-3. Pedigree of Fla. 8326. (Fla. 7708 contains H7998 in its pedigree.)

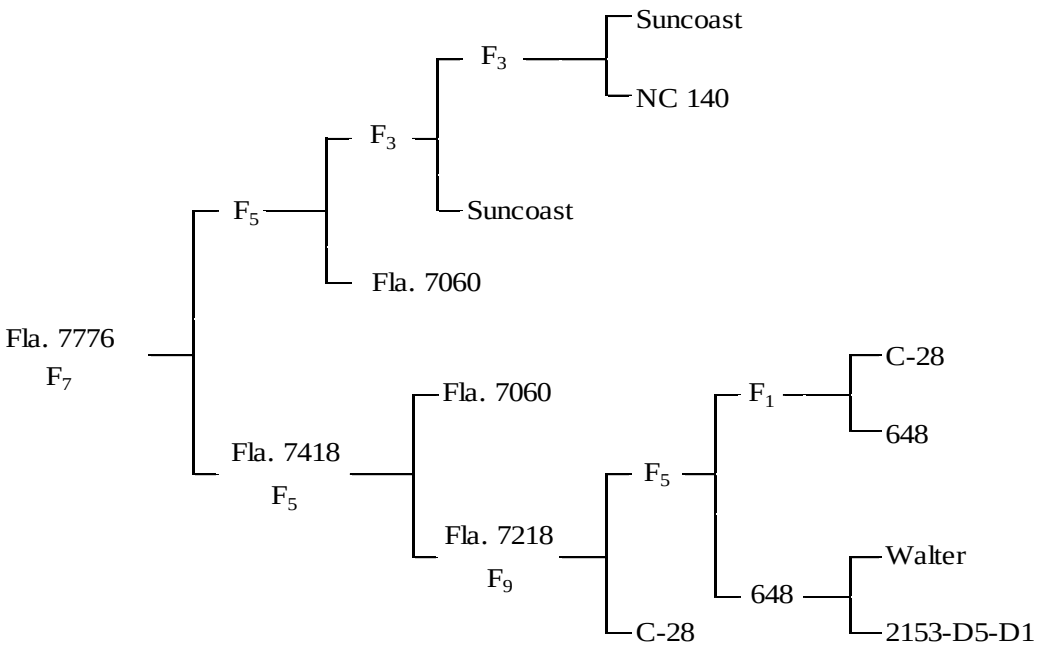


Figure A-4. Pedigree of Fla. 7776.

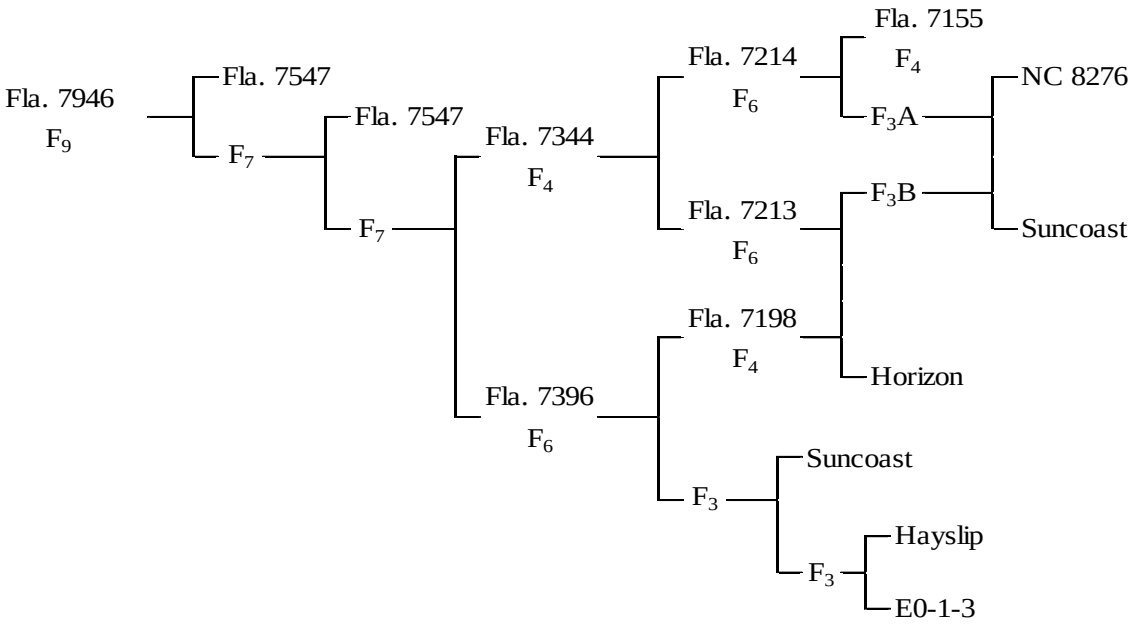


Figure A-5. Pedigree of Fla. 7946.

APPENDIX B
ADDITIONAL MOLECULAR MARKER INFORMATION

Table B-1. Markers screened by a modified EcoTILLING approach to identify polymorphisms between PI 114490 and Florida 7776.

Marker	Approximate position[z]	Polymorphism identified	Status
C2_At5g37360	4.056	yes	verified by sequencing, screened
LE_0107A05CM1	6.003	yes	verified by sequencing, screened
C2_At1g07080	6.004	yes	verified by sequencing, screened
Hba0037I09CM5	3.005	yes	verified by sequencing, screened
Hba0185J10CM6	6.005	yes	verified by sequencing, screened
C8B	6.005	yes	verified by sequencing, screened
Hba0019E05P6-6	6.006	yes	verified by sequencing, screened
CT119	6.006	yes	verified by sequencing, screened
U225722	6.010	yes	verified by sequencing, screened
C2_At3g10920[y]	6.013	yes	verified by sequencing, screened
SCBC 792	6.075	yes	verified by sequencing, screened
cLES-1-K3	6.090	yes	verified by sequencing, screened
TG216-1	7.062	yes	verified by sequencing, screened
U229378[y]	8.017	yes	verified by sequencing, screened
C2_At2g41680[y]	9.016	yes	verified by sequencing, screened
TG523[y]	11.029	yes	verified by sequencing, screened
C2_At4g22260	11.037	yes	verified by sequencing, screened
CT182	11.039	yes	verified by sequencing, screened
cLEX-4-G10	11.044	yes	verified by sequencing, screened
cTOE-14-L16	11.060	yes	verified by sequencing, screened
TG384	11.066	yes	verified by sequencing, screened
C2_At1g30825	11.072	yes	verified by sequencing, screened
C2_At3g54470	11.072	yes	verified by sequencing, screened
cLET-24-J2	11.094	yes	verified by sequencing, screened
U214980	6.005	yes	verified by sequencing, not screened
Mi23	6.006	yes	verified by sequencing, not screened
Rex	6.006	yes	verified by sequencing, not screened
C2_At4g34215	6.069	yes	verified by sequencing, not screened
MboI0011K15CMTY4C	6.093	yes	verified by sequencing, not screened
C2_At1g44446	11.041	yes	verified by sequencing, not screened
cLPT-2-E21-1	3.061	possible	not verified
TG593	6.040	possible	not verified
TG637	7.043	possible	not verified
T1682	10.066	possible	not verified
TG83	1.100	no	n/a[x]
C2_At3g04870[y]	1.102	no	n/a[x]
C2_At4g29120[y]	1.112	no	n/a[x]
C2_At1g56345	1.116	no	n/a[x]
TG465-F1,R2	1.116	no	n/a[x]
U151109	1.117	no	n/a[x]
T0276	1.124	no	n/a[x]
TG528	1.128	no	n/a[x]
C2_At1g10240[y]	1.128	no	n/a[x]
TG269	1.130	no	n/a[x]
C2_At4g22200[y]	1.132	no	n/a[x]
HBa0044E20[y]	1.133	no	n/a[x]
C2_At5g64350[y]	1.137	no	n/a[x]
HBa0010D01[y]	1.140	no	n/a[x]

Table B-1. Continued

Marker	Approximate position[z]	Polymorphism identified	Status
C2_At2g14910	1.151	no	n/a[x]
T0562	2.071	no	n/a[x]
C2_At4g35560[y]	2.079	no	n/a[x]
T1494[y]	2.080	no	n/a[x]
C2_At5g66090[y]	2.083	no	n/a[x]
C2_At5g66530[y]	2.088	no	n/a[x]
HBa0104A12[y]	2.118	no	n/a[x]
C2_At5g63460[y]	3.083	no	n/a[x]
C2_At1g05350[y]	3.085	no	n/a[x]
C2_At5g49970[y]	3.100	no	n/a[x]
T0707[y]	4.000	no	n/a[x]
C02HBa0079G02[y]	4.012	no	n/a[x]
C2_At1g68100[y]	4.013	no	n/a[x]
U234506[y]	4.016	no	n/a[x]
T0208	4.019	no	n/a[x]
TG370-1	4.022	no	n/a[x]
C2_At4g25650[y]	4.037	no	n/a[x]
T0635	4.055	no	n/a[x]
T0819-1	4.070	no	n/a[x]
T0819-3	4.070	no	n/a[x]
T0883-1	4.070	no	n/a[x]
C2_At1g71810[y]	4.072	no	n/a[x]
T1405[y]	4.077	no	n/a[x]
HBa 255I02.1[y]	4.080	no	n/a[x]
C2_At4g09010[y]	4.083	no	n/a[x]
C04HBa0053M02[y]	4.088	no	n/a[x]
C2_At1g60440	5.000	no	n/a[x]
C2_At1g60200	5.007	no	n/a[x]
C2_At5g14320	5.010	no	n/a[x]
TG598-1	5.012	no	n/a[x]
P11M6[y]	5.015	no	n/a[x]
C2_At2g01110[y]	5.037	no	n/a[x]
C2_At1g13380[y]	5.039	no	n/a[x]
TG626[y]	5.043	no	n/a[x]
C2_At1g07040[y]	5.047	no	n/a[x]
C2_At2g03510[y]	5.055	no	n/a[x]
C2_At1g67700	5.067	no	n/a[x]
C2_At3g55800[y]	5.090	no	n/a[x]
HBa 0246L12CM12	6.003	no	n/a[x]
TG297	6.004	no	n/a[x]
T1636	6.005	no	n/a[x]
32.5 Cla	6.006	no	n/a[x]
CT21	6.009	no	n/a[x]
TG436	6.010	no	n/a[x]
TG221	6.010	no	n/a[x]
U231369	6.013	no	n/a[x]

Table B-1. Continued

Marker	Approximate position[z]	Polymorphism identified	Status
C2_At3g56130[y]	6.017	no	n/a[x]
U219302	6.018	no	n/a[x]
TG118L	6.022	no	n/a[x]
U215559	6.024	no	n/a[x]
C2_At3g11210	6.025	no	n/a[x]
C2_At5g05690	6.025	no	n/a[x]
PG 8	6.025	no	n/a[x]
T0507-F2R1	6.025	no	n/a[x]
PG10	6.025	no	n/a[x]
PG 5	6.025	no	n/a[x]
PG 9	6.025	no	n/a[x]
R end	6.025	no	n/a[x]
C2_At5g41480	6.026	no	n/a[x]
T1079	6.027	no	n/a[x]
C2_At4g27700[y]	6.027	no	n/a[x]
T1098	6.030	no	n/a[x]
U161340 (TG 240)	6.038	no	n/a[x]
CT58	6.038	no	n/a[x]
CT184	6.038	no	n/a[x]
T0805	6.043	no	n/a[x]
T1666	6.044	no	n/a[x]
TG472	6.044	no	n/a[x]
C2_At5g07960	6.052	no	n/a[x]
C2_At5g62530	6.056	no	n/a[x]
CT174	6.056	no	n/a[x]
TG552 F2R2	6.057	no	n/a[x]
C2_At4g24690	6.063	no	n/a[x]
C2_At1g24360	6.063	no	n/a[x]
TG578	6.064	no	n/a[x]
C2_At1g09340	6.067	no	n/a[x]
C2_At3g11710	6.068	no	n/a[x]
TG275	6.069	no	n/a[x]
TG279	6.073	no	n/a[x]
U312958	6.074	no	n/a[x]
TG314	6.083	no	n/a[x]
Hba0169D11CMTY4A	6.085	no	n/a[x]
TG642-rev	6.090	no	n/a[x]
TG215-for	6.093	no	n/a[x]
TG581	6.096	no	n/a[x]
Hba 0217M17CMTY4B	6.097	no	n/a[x]
TG115	6.097	no	n/a[x]
U146140	6.097	no	n/a[x]
B101L7M2	6.[w]	no	n/a[x]
M1349	6.[w]	no	n/a[x]
M2147	6.[w]	no	n/a[x]
U216282	6.[w]	no	n/a[x]
U226440	6.[w]	no	n/a[x]
U234831	6.[w]	no	n/a[x]
U 213367	6.[w]	no	n/a[x]

Table B-1. Continued

Marker	Approximate position[z]	Polymorphism identified	Status
Sp2 F1R1	6.[w]	no	n/a[x]
Spr2 F2R2	6.[w]	no	n/a[x]
U231937	6.[w]	no	n/a[x]
U146482	6.[w]	no	n/a[x]
B ronen	6.[w]	no	n/a[x]
H9A11-con	6.[w]	no	n/a[x]
U212715	6.[w]	no	n/a[x]
TG342	7.000	no	n/a[x]
C2_At5g56940	7.011	no	n/a[x]
C2_At2g06925	7.019	no	n/a[x]
C2_At4g29490[y]	7.022	no	n/a[x]
FW7[y]	7.024	no	n/a[x]
C2_At3g13050[y]	7.028	no	n/a[x]
U242881[y]	7.030	no	n/a[x]
C2_At3g04600	7.037	no	n/a[x]
TG202	7.037	no	n/a[x]
C2_At5g14520[y]	7.038	no	n/a[x]
TG217[y]	7.043	no	n/a[x]
CT84	7.043	no	n/a[x]
T1329	7.043	no	n/a[x]
C2_At2g42750	7.044	no	n/a[x]
C2_At4g03210	7.045	no	n/a[x]
TG572	7.048	no	n/a[x]
U216327[y]	7.052	no	n/a[x]
C2_At1g53670[y]	7.054	no	n/a[x]
CT54[y]	7.058	no	n/a[x]
TG143	7.060	no	n/a[x]
TG183	7.063	no	n/a[x]
T1738[y]	7.073	no	n/a[x]
C2_At4g26750	7.080	no	n/a[x]
T1255	7.082	no	n/a[x]
TG128	7.085	no	n/a[x]
C2_At1g55870	7.090	no	n/a[x]
C2_At5g56130	7.108	no	n/a[x]
C2_At5g46630[y]	8.000	no	n/a[x]
HBa0025I17-1[y]	8.004	no	n/a[x]
HBa0025I17-2[y]	8.004	no	n/a[x]
C2_At5g11480[y]	8.039	no	n/a[x]
C2_At3g43540[y]	8.041	no	n/a[x]
HBa0076J13.1[y]	8.047	no	n/a[x]
TG505[y]	8.053	no	n/a[x]
C2_At5g47010[y]	8.056	no	n/a[x]
C2_At2g37240	9.001	no	n/a[x]
TG18[y]	9.014	no	n/a[x]
C09HBa0116C14-1[y]	9.014	no	n/a[x]
C2_At2g36930[y]	9.015	no	n/a[x]
C2_At2g37025[y]	9.015	no	n/a[x]

Table B-1. Continued

Marker	Approximate position[z]	Polymorphism identified	Status
HBa0203J14-1[y]	9.023	no	n/a[x]
C2_At3g09925[y]	9.025	no	n/a[x]
C2_At3g13235	10.000	no	n/a[x]
C2_At5g06430[y]	10.003	no	n/a[x]
TG303[y]	10.011	no	n/a[x]
CT203[y]	10.041	no	n/a[x]
C2_At3g54360[y]	10.056	no	n/a[x]
CT20	10.058	no	n/a[x]
C2_At3g12290[y]	10.058	no	n/a[x]
C2_At3g09740	10.072	no	n/a[x]
TG497	11.000	no	n/a[x]
T0408	11.026	no	n/a[x]
C2_At5g16710[y]	11.031	no	n/a[x]
C2_At1g44790	11.041	no	n/a[x]
T0675	11.042	no	n/a[x]
cLEB 7L1	11.046	no	n/a[x]
TG109	11.047	no	n/a[x]
TG44	11.047	no	n/a[x]
TG47	11.049	no	n/a[x]
CT55	11.051	no	n/a[x]
TG147	11.053	no	n/a[x]
C2_At4g10050	11.054	no	n/a[x]
TG110	11.054	no	n/a[x]
TG400[y]	11.057	no	n/a[x]
C2_At3g44880	11.061	no	n/a[x]
C2_At2g27730	11.069	no	n/a[x]
C2_At3g52730	11.070	no	n/a[x]
C2_At2g27450	11.073	no	n/a[x]
TG36	11.083	no	n/a[x]
TG393	11.097	no	n/a[x]
T10	11.[w]	no	n/a[x]
CT79	12.033	no	n/a[x]
CT99[y]	12.045	no	n/a[x]
TG283	12.055	no	n/a[x]
TG618	12.055	no	n/a[x]
T0801	12.074	no	n/a[x]
TG28	12.[w]	no	n/a[x]
LE_HBa0088F22	not mapped	no	n/a[x]

[z] Number represents chromosome and map position (in cM).

[y] Marker was also screened for polymorphisms by restriction digestions

[x] Markers identified as non-polymorphic were not further investigated.

[w] Precise map position has not been determined.

Table B-2. Technical information for markers polymorphic among genotypes resistant or susceptible to bacterial spot.

Marker	Approximate position[z]	Forward primer	Reverse primer	Annealing temperature	Amplicon size(s)	Restriction enzyme	Detection
SSR478	1.000	gcagcatatatcaccttggct	cgtgctctccaatagttcacc	50	450, 400	n/a	4% agarose
Cf9	1.005	caggcacagagttacatggg	caaccagtgaaggaagggag	60	600	*Hae* III	2% agarose
CosOH47	1.010	ttgctgattttcttcccatttt	gcagctggagtgagaggaac	54	226	*Bst* UI	2% agarose
LEOH36	1.019	gcagcatatatcaccttggct	cgtgctctccaatagttcacc	54	1300	*Bcl* I	2% agarose
C01HBa0003D15.1	1.029	tctcccacgagcaatcggaagaat	tttcagtggcctcaagctctcaca	55	1100	*Alu* I	2% agarose
C2_At5g18580	1.035	tgccacattgcctctgtatgtacagaac	atgtcaattcgggcttgagtaagtg	49	920	*Hpy* CH4IV	2% agarose
C2_At5g18580	1.035	tgccacattgcctctgtatgtacagaac	atgtcaattcgggcttgagtaagtg	49	920	*Dde* I	4% agarose
CT20134I	1.041	unavailable[y]	unavailable[y]	55	169, 176	n/a	4% agarose
CT10975I	1.051	unavailable[y]	unavailable[y]	55	151, 161	n/a	4% agarose
CT10030L1	1.058	unavailable[y]	unavailable[y]	55	321, 193	n/a	3% agarose
CT20116	1.059	unavailable[y]	unavailable[y]			n/a	Luminex
CT10811	1.065	unavailable[y]	unavailable[y]			n/a	Luminex
CT10629	1.065	unavailable[y]	unavailable[y]			n/a	Luminex
CT10945	1.065	unavailable[y]	unavailable[y]			n/a	Luminex
SSR134	1.075	ccctcttgcctaaacatcca	cgttgcgaattcagattagttg	50	151, 167, 169	n/a	6.5% polyacrylamide
TOM202	1.082	tggtcaccttcaacttttatac	aaatgataatgaaatggagtga	45	196, 202, 204, 210	n/a	6.5% polyacrylamide
C2_At3g04710	1.095	agggtgcagatcctgcaatacccag	tccagcctcactttgtaaatcaacatc	55	1500	*Hinc* II	2% agarose
LEOH106	1.095	agggagaaatttgacatacgg	ggaccaacagcaaatacaaaa	52	258	*Alu* I	2% agarose
TG59	1.097	tgcacacccttttctttgattc	tcagttctatcaagtccatccac	55	1800	*Dpn* II	2% agarose
TG59	1.097	tgcacacccttttctttgattc	tcagttctatcaagtccatccac	55	1800	*Mnl* I	4% agarose
LEVCOH11	1.100	caaccatgttagatgtgccagt	taagagaggggaatggtgatgt	54	149	*Mnl* I	4% agarose
LEVCOH12	1.101	ggagaaagaagatccatcaaagg	attaaaacaacagaagagaaaccag	54	100	BsaJ I	4% agarose
C2_At3g04870	1.102	acgcgtgctagcatccagagg	tgacatggcaagcccactaacatac	55	450	*Hpy* CH4IV	2% agarose
U237757	1.102	atcggctgctgatgtttatgatcg	acaacatccctccatagagtttcaag	55	2000	*Mnl* I	2% agarose
C2_At1g02560	1.116	tttatcttgatgctgttgatcccac	tgaccctcctggagaattgacatac	57	750	n/a	2% agarose
SSR308	1.116	tttccctgtttcagcctttg	ggcacgagaatttagccact		291, 301	n/a	6.5% polyacrylamide
C2_At5g49880	1.127	tcggctccgttgccggaatc	actgcttaaggcatcaaaaaactc	55	900	*Rsa* I	2% agarose
LE_HBa0044E20	1.128	tttgatacaagctacgccgggact	tctgcaagtagatgggttactagg	54	450	*Hpy* CH4IV	2% agarose
C2_At4g14110	1.136	agctccttctttccgcttattcaac	tggaagacatattcagtcaagcgctg	55	1100	*Hinc* II	2% agarose
SSR65	1.153	ggcaggagattggttgctta	ttcctcctgtttcatgcattc	50	228, 232	n/a	6.5% polyacrylamide

Table B-2. Continued

Marker	Approximate position[z]	Forward primer	Reverse primer	Annealing temperature	Amplicon size(s)	Restriction enzyme	Detection
LEOH342	2.000	tcctttgattgtttcaacacc	tccacaactccctgaaaagg	52	208, 209	n/a	6.5% polyacrylamide
TOM11	2.014	attgtaatggtgatgctcttcc	cagttactaccaaaaatagtcaaacac	45	183, 187	n/a	6.5% polyacrylamide
CT10682I	2.029	unavailable[y]	unavailable[y]	55	176, 182	n/a	4% agarose
SSR66	2.031	tgcaacaactggataggtcg	tggatgaaacggatgttgaa	50	116, 125, 128	n/a	6.5% polyacrylamide
SSR104	2.037	ttccatttgaattccaaccc	cccactgcacatcaactgac	50	742, 777	n/a	4% agarose
SSR96	2.039	gggttatcaatgatgcaatgg	cctttatgtcagccggtgtt	50	199, 209, 221	n/a	6.5% polyacrylamide
LEOH23.3	2.042	ctatgcgtttgtcggtcgt	caaggtagttgaaggtatgacca	54	155	*Tsp* 509I	2% agarose
CT10649	2.042	unavailable[y]	unavailable[y]			n/a	Luminex
SSR5	2.044	tggccggcttctagaaataa	tgaaatcacccgtgaccttt	50	190, 193, 196	n/a	6.5% polyacrylamide
SSR349A	2.044	gagtgatcatccatcctctca	ggaagagactttggactaaggga	50	219, 231, 239	n/a	6.5% polyacrylamide
CT10923	2.044	unavailable[y]	unavailable[y]			n/a	Luminex
CT10153	2.048	unavailable[y]	unavailable[y]			n/a	Luminex
CT10771	2.048	unavailable[y]	unavailable[y]			n/a	Luminex
CT10801	2.051	unavailable[y]	unavailable[y]			n/a	Luminex
CT10279I	2.055	unavailable[y]	unavailable[y]	55	160, 166	n/a	4% agarose
TOM188	2.059	cccacctttttacctctccc	ggaagatggtatttttggaaa	45	159, 163, 167	n/a	6.5% polyacrylamide
LEOH348	2.072	tgtttcccttcattcatgct	ccaattggataaattggtggt	52	150	*Hpy* CH4IV	2% agarose
CosOH7	2.074	catggatatggtaattggagga	ccttttcctgatatgcgtattcc	54	502	*Hinf* I	2% agarose
HBa44O16SP6	2.075	cttgttggcaatgcaagaga	aaggccgtgaatcattgaac	55	500	*Hae* III	2% agarose
C2_At4g35560	2.079	agttcaacacgtgtagcttgcatgg	aacagatcaatactaatggttgctttg	50	350	*Tsp* 509I	2% agarose
C2_At5g66090	2.083	atctctctgagggttcaagacagg	tatatcagctccatacttctttgc	55	1200	*Rsa* I	2% agarose
C2_At5g66090	2.083	atctctctgagggttcaagacagg	tatatcagctccatacttctttgc	55	1200	*Hpy* CH4III	2% agarose
LEOH319	2.107	tgcaataaggcctgatacgg	tgcccacttaacgacatcaa	52	200	*Tsp* 509I	4% agarose

Table B-2. Continued

Marker	Approximate position[z]	Forward primer	Reverse primer	Annealing temperature	Amplicon size(s)	Restriction enzyme	Detection
CT10690I	3.003	unavailable[y]	unavailable[y]	55	157, 170	n/a	3.5% agarose
CT10772I	3.004	unavailable[y]	unavailable[y]	55	176, 182	CelI	2% agarose
CT20050	3.004	unavailable[y]	unavailable[y]			n/a	Luminex
CT201821	3.013	unavailable[y]	unavailable[y]	55	223, 227	CelI	2% agarose
CT20182	3.013	unavailable[y]	unavailable[y]	55	223, 227	CelI	2% agarose
CT10480I	3.038	unavailable[y]	unavailable[y]	55	124, 144	n/a	3% agarose
LEOH223	3.040	acaagagtcgggtgatggac	gcgatggaaatagcatcaca	52	177	*Mse* I	2% agarose
CT10402I	3.046	unavailable[y]	unavailable[y]	45	250, 255	n/a	6.5% acrylamide
CT20195	3.046	unavailable[y]	unavailable[y]			n/a	Luminex
T1388	3.047	gcgatttggctatctgggta	aaccgaaaggcttttccaag	55	1000	*Mnl* I	2% agarose
T1388	3.047	gcgatttggctatctgggta	aaccgaaaggcttttccaag	55	1000	*Hpy* CH4IV	2% agarose
CT20037	3.047	unavailable[y]	unavailable[y]			n/a	Luminex
CT10736	3.050	unavailable[y]	unavailable[y]			n/a	Luminex
LEOH124SNP	3.059	ccgtctccttctccctcttt	ctggctggtgtcttctccat	52	208	*Hha* I	2% agarose
LEGTOM5c	3.064	tttagccgtgttgtgaaatcc	gactttcaaaaggcatccgtc	56	142	*Mse* I	2% agarose
SSR111	3.070	ttcttcccttccatcagttct	tttgctgctatactgctgaca		197, 201, 205	n/a	6.5% polyacrylamide
C2_At1g02140	3.071	tccgttatgctaacaattccaac	tgtgttcatttcccatcacaatctc	50	1500, 1550	*Hha* I	2% agarose
SSR231	3.075	tgccaatccactcagacaaa	tggattcaccaaggcttctt	50	165, 167	n/a	6.5% polyacrylamide
C2_At5g62390	3.076	tgctactaactgttgatgccattgag	ttgggggtcgataacatcaagc	55	1200	*Hinf* I	2% agarose
LEOH185	3.078	cgtcacagtcgcgtaaatga	ccttcttccccaatttcctc	52	159, 169	n/a	6.5% polyacrylamide
FEY	3.079	accgcttcctctatcaagca	atgccgaataaccaagcaac	55	667	*Bst* UI	2% agarose
C2_At5g63460	3.083	ttctcgcggccttttctcctc	tcgtgatcgcaaacatatactcgc	53	1100	*Alu* I	2% agarose
C2_At5g60160	3.083	acacaatgctaatcaacgttatgc	tcatccaccgcgcacatttc	54	470	*Hinf* I	4% agarose
C2_At1g05350	3.085	tgaacgaaccctaaagcgtgaagg	tccgaacttcaacaagtacttcaatgtg	55	530	*Dde* I	2% agarose
C03HBa0082F22	3.087	cacattccacctataaacatcaacgtacag	ggtgattgagtaatttcccttagtcttc	55	600	*Hinc* II	2% agarose
C2_At5g52820	3.090	tgggatctaaatacccagacacc	acagaaagaacccaatttctgtgc	55	750	*Hpy* CH4IV	2% agarose
U146899	3.097	acttgaccgggaaagtgatg	ccgatcgtcttctccattgt	53	1700	*Hinc* II	2% agarose
C2_At5g49970	3.100	aattggcaggcttgagtgttgc	tcccaccattgttaccaggaccac	53	580, 750	*Rsa* I	2% agarose
C2_At3g47990	3.102	agagaagcagtggaggcactcattc	agaaaaccttgcaacctcagcag	55	1100	*Aci* I	2% agarose
C2_At1g61620	3.106	atgcattctagaatgccttttgtc	tccctggctttctgcagcatc	54	1600	*Taq* I	2% agarose
CosOH51	3.107	ctcatttgatacctctatttgtggtg	tgagatcttaaaagaaacacatgagg	56	288	*Rsa* I	2% agarose
SSR320	3.112	atgaggcaatcttcacctgg	ttcagctgatagttcctgcg	50	166, 170, 172	n/a	6.5% polyacrylamide
SSR601	3.112	tctgcatctggtgaagcaag	ctggattgcctggttgattt	50	158, 164, 167	n/a	6.5% polyacrylamide
LEOH127	3.113	caaggcatcaacctaattgga	tgtaggcttgaaaaataagaggaga	52	244	*Hinc* II	2% agarose
CT10494	3.113	unavailable[y]	unavailable[y]			n/a	Luminex

Table B-2. Continued

Marker	Approximate position[z]	Forward primer	Reverse primer	Annealing temperature	Amplicon size(s)	Restriction enzyme	Detection
Hero	4.007	cagcatctttcaggcaaaca	ggagttcgttcgctctttg	55	1111	*Mnl* I	2% agarose
Hero	4.007	cagcatctttcaggcaaaca	ggagttcgttcgctctttg	55	1111	*Mwo* I	2% agarose
TG15-2	4.012	tcatccgctcccaatagtcatcca	gtgggtgttcggttcttcagttca	53	1850	*Hpy* CH4IV	2% agarose
TOM194	4.014	acgaagtaatacagccaatg	agccatccaacacaaaacac	45	202, 206	n/a	6.5% polyacrylamide
SSR43	4.015	ctccaaattgggcaataaca	ttaggaagttgcattaggcca	50	234, 237	n/a	6.5% polyacrylamide
C2_At3g17040	4.022	tggggttggatggagtggaaag	agtagaggttacgaatttcctctgc	55	470	*Dde* I	2% agarose
CT20145	4.041	unavailable[y]	unavailable[y]			n/a	Luminex
CT188-2	4.042	aagctcatgtgaaacagaccacgc	actgtgaacaactccgatctgcct	48	1500	*Hha* I	2% agarose
TG182	4.046	ttttctgaatggtttactctggaa	ttatgggatgacagcaagca	55	450	*Rsa* I	2% agarose
CT10184	4.047	unavailable[y]	unavailable[y]			n/a	Luminex
C2_At5g37360	4.056	tgcagcatttgatctttcaaatgg	tgatttttgagagcctgtcaatgag	50	1282	*Mae* III	2% agarose
LEOH37	4.068	ttgatatattccatgtgtgtctc	aactacaaattaacaaacttaaatgg	51	190	*Tsp* 45I	2% agarose
C2_At1g71810	4.072	tcatgcagatccacatcctggaaac	agtgacaaaatccttggccaatgc	57	1050	*Bst* UI	2% agarose
CT185	4.076	tcggacagaggttgataacacagc	agaagcaatcataaagggtcgcca	54	350	*Alu* I	2% agarose
CT10888	4.078	unavailable[y]	unavailable[y]	55	266	n/a	4% agarose
CT101841	4.079	unavailable[y]	unavailable[y]	55	130, 142	n/a	3.5% agarose
C2_At1g27530	4.088	tgttaacaatcaaagctggtccacg	tgcccatagcggcttgaaatgc	55	620	*Dpn* II	4% agarose
TG500	4.098	aggcaacctgcacaaacaagaacc	gttagccgccgtcacaagtaaagt	55	1150	*Dde* I	2% agarose
TG500	4.098	aggcaacctgcacaaacaagaacc	gttagccgccgtcacaagtaaagt	55	1150 +375	n/a	2% agarose
LEOH10	4.114	tgccagattgactgtgaagg	ggaaccctgcattgttcttg	55	200	*Bsa* JI	2% agarose

Table B-2. Continued

Marker	Approximate position[z]	Forward primer	Reverse primer	Annealing temperature	Amplicon size(s)	Restriction enzyme	Detection
C05HBa0261K11	5.007	gctgaaaccaatcgccaaccatca	attgccagtgaagaggccaagaga	55	1120	*Tsp* 509I	2% agarose
TG441	5.008	tccaagcctgctctgaggtaa	cagcttgaactgtgtcatgtaac	50	1000	*Taq* I	2% agarose
P11M6	5.015	gaggtaggacttagaaaacata	aatcaacaccactaaatgcaga	50	700	*Taq* I	4% agarose
C2_At1g26945	5.018	atcgctgatcttgtttccaagttgc	aatagcagcttgagcactatcactatc	53	1800	*Dpn* II	2% agarose
Bs4	5.022	ggagctgaatacggattgga	atcgttccgatgatttctgg	55	1100	*Dpn* II	2% agarose
CT93	5.037	ttctgaggttggctgagaccttgt	tctggtagacaatggaaccgcctt	54	750	*Alu* I	2% agarose
C2_At1g13380	5.039	aggtgctttcttgtttcttctttc	agagcatatcacgatacttggtgtg	55	800	*Hpy* 188I	2% agarose
CT202101	5.045	unavailable[y]	unavailable[y]	45	157, 175	n/a	6.5% acrylamide
C2_At4g24830	5.051	atacttgcttgggacatcaatggc	tccaacctctctggcaacatcaacc	55	1400	*Hpy* CH4IV	2% agarose
C2_At4g24830	5.051	atacttgcttgggacatcaatggc	tccaacctctctggcaacatcaacc	55	1400	*Tsp* 509I	4% agarose
TOM152	5.058	attcaaggaacttttagctcc	tgcattaaggttcataaatga	45	208, 210, 230	n/a	6.5% polyacrylamide
C2_At1g14000	5.060	agcgttacatggctggatcgatg	atacgtctttaacaattcaatcatgc	55	660	*Spe* I	2% agarose
TOM49	5.068	aagaaacttttttgaatgttgc	attacaatttagagagtcaagg	45	223, 203, 191	n/a	6.5% polyacrylamide
CT10591	5.076	unavailable[y]	unavailable[y]			n/a	Luminex
LEOH316	5.078	ctccgagcgaagagtctagagtc	gaaggcaaaaggaaaaggagaaggatgg	52	152, 142	*Mbo* II	4% agarose
Rx3-L1	5.079	ctccgagcgaagagtctagagtc	gaaggcaaaaggaaaaggagaaggatgg	61	323	*Bsr* BI	2% agarose
CosOH73	5.082	cttcccgacaagcacaaaaa	cgaatgctctgtaccatttcc	56	123	*Alu* I	2% agarose
C2_At3g55800	5.090	tttgaaatcaagctcattatttgg	agctgttcctccacaagaagctg	55	350	*Hinc* II	2% agarose
TG351	5.094	cagaaacaggaacacaggacaagg	aggctcacaggaacggaatcaaga	55	988	*Mwo* I	2% agarose

Table B-2. Continued

Marker	Approximate position[z]	Forward primer	Reverse primer	Annealing temperature	Amplicon size(s)	Restriction enzyme	Detection
C06HBa0107A05	6.003	gaaggatttagaagtgtaggaac	gcggtttgattcgattttgtac	55	500	*Mbo* II	2% agarose
C2_At1g07080	6.004	ttgtgccatgggttgttgttgatg	tgtaagctttgcaaatgtagcttatg	55	644, 626	*Ban* I	2% agarose
C06HBa0185J10	6.005	cgaagaacaatgtgaaggtttgac	cttgaaacaaatctcaccacatag	56	420	*Bsm* FI	2% agarose
LE_HBa0037I09	6.005	gttacttgatgcttaattattgatgtc	gcagtgcagtcatgaaagccac	55	541, 554	*Bse* RI	2% agarose
C06HBa0019E05	6.006	caatttataggtgtttttgggacatc	gttcaacacttggccaatgcttacg	56	650	*Apo* I	2% agarose
C8B	6.006	tacccacgccccatcaatg	tgcaagagggtgaatattgagtgc	55	407	*Hha* I	2% agarose
CT119	6.006	actattctcacgtaaggggacac	gtgtacatgtatgaaactctagc	55	316	*Rsa* I	2% agarose
SSR47	6.007	tcctcaagaaatgaagctctga	ccttggagataacaaccacaa	50	172, 189, 195	n/a	6.5% polyacrylamide
CT10242I	6.007	unavailable[y]	unavailable[y]	55	163, 184	n/a	3% agarose
CT10242	6.007	unavailable[y]	unavailable[y]			n/a	Luminex
TG97	6.009	caccacataattgagaaggacaacac	catcattgctattgaagtcatccg	55	375	n/a	2% agarose
T1456	6.010	tagcttctgccattgatttgagc	tgagagggaagtatctgtatgccc	56	650	*Rsa* I	2% agarose
CT10187	6.011	unavailable[y]	unavailable[y]			n/a	Luminex
C2_At3g10920	6.013	tggcttggtgtggacaaagagc	tgcaagtagtatgcgtgttccc	55	650	*Dpn* II	2% agarose
C2_At3g10920	6.013	tggcttggtgtggacaaagagc	tgcaagtagtatgcgtgttccc	55	682	*Alu* I	2% agarose
C2_At3g56130	6.017	tgtttgcctcggtttctccgatca	cataaccaacagcctcgccatcat	55	625	*Hpy* CH4III	2% agarose
TG352	6.018	gcactacagcacaaccgcataagt	actaaagagtaaagacacacagattc?	50	625	*Taq* I	2% agarose
TG590 F2R2	6.022	acagcaggaggtgatggaatac	cgggtcgagcgatttgttta	55	800	*Hpy* CH4III	2% agarose
FLUW25	6.025	caagtgtgcatatacttcatattcac?	ccatatataacctctgtttctatttcg?	55	450	*Taq* I	2% agarose
P6-25F2R5	6.025	ggtagtggaaatgatgctgctc	gctctgcctattgtcccatatataacc	55	290	*Taq* I	2% agarose
CT10328I	6.027	unavailable[y]	unavailable[y]	55	234, 246, 256	n/a	4% agarose
T0834-F1a,R2	6.032	ctgttaattgggaccccatcagaagcagg	ggaaggtgatgctgcaatccttcagataacc	55	550, 600	n/a	3% agarose
C2_At1g44760	6.040	ttcttcatctgctgctcatcttgc	agagggtttttctgacccaagac	55	750	*Alu* I	2% agarose
C2_At1g44760	6.040	ttcttcatctgctgctcatcttgc	agagggtttttctgacccaagac	55	750	*Mse* I	2% agarose
C2_At1g44760	6.040	ttcttcatctgctgctcatcttgc	agagggtttttctgacccaagac	55	759,765	*Nsi* I	2% agarose
TG356	6.044	gcactacagcacaaccgcataagt	gcatccttgaggtcgataacagca	55	763	*Taq* I	2% agarose
TG435	6.058	gccgcatgaagcctaaacaaatcc	tccctgacaattacgagagaccca	55	440	n/a	4% agarose
SP	6.066	agggttgaagttcatggtgg	gatgttccctgagatatgga	56	370	*Bst* NI	4% agarose
LEOH200	6.069	gggtttatgttggtgatatggtg	tcagcagctaaaagtcgaacc	52	150	*Eco* RV	2% agarose
LEOH112	6.072	gccaattgaactgaccatctg	cccatgtatttggctgtagaa	52	250	*Hpy* CH4IV	2% agarose
SCBC 792	6.075	caacccacactaggcaagtcgggt	caacccacaccccatttttta	56	900	CelI	2% agarose
cLES-1-K3	6.090	tcacagagacaaccaagcaatccc	cttacaaccatcatcatccagaacg	55	900	*Age* I	2% agarose

Table B-2. Continued

Marker	Approximate position[z]	Forward primer	Reverse primer	Annealing temperature	Amplicon size(s)	Restriction enzyme	Detection
CT20017	7.003	unavailable[y]	unavailable[y]			n/a	Luminex
C2_At5g20180	7.006	tgctatgtacatctaatcccaagcac	agctatccccctttccaccaag	55	1300	*Bst* UI	2% agarose
C2_At5g20180	7.006	tgctatgtacatctaatcccaagcac	agctatccccctttccaccaag	55	1300	*Taq* I	2% agarose
C2_At2g26590	7.015	agccagcagctgacaatgatgcac	tggtaaaggagctgaagcttcagg	55	900	CelI	2% agarose
C2_At4g29490	7.022	aagagcaaactcgacattgcacc	acaagtaggcgaaatagctctcctg	54	530	*Rsa* I	2% agarose
C2_At1g19140	7.024	aggcccttgtactcagtgcctctc	tcatggcggtttcagtccatcc	55	1100, 855	n/a	2% agarose
FW7	7.024	acagccagacccttctcatact	ggatcctaaaagaatgtgcagt	55	725, 450, 350	*Taq* I	2% agarose
SSR276	7.037	ctccggcaagagtgaacatt	cgacggagtacttcgcattt	52	148, 150, 177	n/a	4% agarose
C2_At4g26680	7.038	tgcaaatcgggaaaactagaaaagg	acagtcccataatcaatgcattgtaag	55	450	*Alu* I	2% agarose
TG217	7.073	cgttgcttcctgatcctacc	agctagtgatgatcctggcg	55	800	*Hpy* CH4IV	4% agarose
C2_At2g20860	7.043	attgaagccacatatactcatagaagc	tccagattttgcaactttctctacac	55	1350	*Bsa* BI+*Bsa*	2% agarose
C2_At2g20860	7.043	attgaagccacatatactcatagaagc	tccagattttgcaactttctctacac	55	1350	*Mwo* I	2% agarose
C2_At2g20860	7.043	attgaagccacatatactcatagaagc	tccagattttgcaactttctctacac	55	1350	*Nla* III	2% agarose
C2_At1g53670	7.054	aagggtacagaacgggcattcac	tgttccaggggtcttactgttccag	55	1300	*Hha* I	2% agarose
TG216-1	7.062	gctttcggtactgcatcctc	taaatgaagcctgggattgc	55	860	*Bsl* I	2% agarose
TG174	7.065	ttccaagatcttttagcgtctc	ctgttgcggatgtgatcatt	55	1500	*Hha* I	2% agarose
LEOH40	7.066	tgagttggtgaaccatggaa	ccaaagttgggacctttga	56	650	*Tsp* 45I	2% agarose
LEOH1.1	7.066	tccacatgaagtaatggacacag	ttcttcgtcaagatcgggta	55	205	*Tsp* 45I	4% agarose
SSR45	7.080	tgtatcctggtggaccaatg	tccaagtatcaggcacacca	50	134, 140, 143	n/a	6.5% polyacrylamide
CT20051	7.[x]	unavailable[y]	unavailable[y]			n/a	Luminex

Table B-2. Continued

Marker	Approximate position[z]	Forward primer	Reverse primer	Annealing temperature	Amplicon size(s)	Restriction enzyme	Detection
C2_At5g46630	8.000	tggcgcctttgatgaagatgc	agattttgagggtaaccaaagtcc	56	850	*Hpy* CH4III	2% agarose
U221657	8.013	aggtttcaatggtggagcac	acagctgctcgtttcagacc	55	750	*Rsa* I	4% agarose
U229378	8.017	aggcagtggttgatatacctttgc	tgttcccatattcatacggtttcc	55	570	*Bst* UI	2% agarose
U229378	8.017	aggcagtggttgatatacctttgc	tgttcccatattcatacggtttcc	55	570	*Hinf* I	2% agarose
LEOH343	8.018	caaatgggtttggctgaaaa	cgcaaactgatttgaacagc	52	132	*Mnl* I	2% agarose
LEOH147	8.019	agttcccgttggtgttcaag	cccttgccagtggatgttag	52	185	*Tsp* 45I	2% agarose
CosOH64	8.020	aagaaatccaatgccaaacggac	cattgccttgacatatccttg	52	115	*Rsa* I	2% agarose
C2_At5g27390	8.021	atggccatgtccacgctcctc	tgggcttagccttatctccatatag	50	900	*Dpn* II	2% agarose
C2_At2g26830	8.030	tcaaatctagatggttctcacttctctg	aagtgcgtgcatcaataaatgactg	50	1500	*Hpy* CH4III	2% agarose
C2_At3g43540	8.041	agcttacatggctaccactactttcac	agaccagaatcaggcaaccctgatg	50	550, 275	*Rsa* I	2% agarose
C2_At3g43540	8.041	agcttacatggctaccactactttcac	agaccagaatcaggcaaccctgatg	50	550	*Aci* I	4% agarose
C2_At3g43540	8.041	agcttacatggctaccactactttcac	agaccagaatcaggcaaccctgatg	50	700+600+430	n/a	2% agarose
C2_At5g25630	8.042	tttcaattcactgatcaaaggtttcc	acctccctgcacttaaccatatatcc	55	1650	*Hpy* CH4IV	2% agarose
TG302	8.044	ctctccgggtggctattaca	tcttgggactcctcctttttct	55	750	*Taq* I	2% agarose
TG302	8.044	ctctccgggtggctattaca	tcttgggactcctcctttttct	55	750	*Alu* I	2% agarose
CosOH42	8.084	ggaattccacatgaagtaatgga	ttgatcaaatcgggcttagg	56	500	*Tsp* 45I	2% agarose

Table B-2. Continued

Marker	Approximate position[z]	Forward primer	Reverse primer	Annealing temperature	Amplicon size(s)	Restriction enzyme	Detection
TG254	9.000	gacttcggggcaattatctg	aaacgagcactgcattcatg	48	1700	n/a	2% agarose
C2_At2g37025	9.015	aacatcacaggctctggactgtttc	atgcatgttcgccagttcactgac	52	1200	*Hpy* CH4IV	2% agarose
C2_At2g37025	9.015	aacatcacaggctctggactgtttc	atgcatgttcgccagttcactgac	52	1200+300	*Taq* I	2% agarose
C2_At2g41680	9.016	tctgtggaaggtgtatttgcagc	agtgatggcttgcctccattc	55	600	CelI	2% agarose
C2_At2g32600	9.017	tgaagggaattacttggctcacac	tgttttgtttccggatcaaattgc	55	1300	*Mwo* I	2% agarose
C2_At3g09920	9.017	aagcaatcataaagggtcacaggag	agaccattgggcaataatctttcc	50	1700	*Dde* I	2% agarose
C09HBa0203J14.1	9.023	gcatgactgctctcagttggcttt	ggcagcttcatttgagtgtggaga	53	845	*Tsp* 509I	2% agarose
C09HBa0203J14.1	9.023	gcatgactgctctcagttggcttt	ggcagcttcatttgagtgtggaga	53	845	*Hinf* I	2% agarose
LEOH31.3	9.039	ttgcaatggcttctctcctc	acttgtccgtttctcgcttg	50	400+240	*Msp* I	2% agarose
SSR383	9.057	attgtacaaagacccgtggc	gttgcacactggatcaatgc	50	187,239	n/a	6.5% polyacrylamide
LEOH144	9.065	atggcctaggattgcatctg	ttgcatacacttggataaaagca	52	225	*Fok* I	2% agarose
TOM236	9.086	gttttttcaacatcaaagagct	ggataggtttcgttagtgaact	45	154,174,188	n/a	6.5% polyacrylamide
SSR333	9.109	gttcccgcttgagaaacaac	ccaatgctgggacagaagat	50	191, 199, 201	n/a	6.5% polyacrylamide
Cosi52	9.[x]	gcctttcttccaggatgcta	cccattttccttcttcctaga	52	192, 226	n/a	4% agarose

Table B-2. Continued

Marker	Approximate position[z]	Forward primer	Reverse primer	Annealing temperature	Amplicon size(s)	Restriction enzyme	Detection
C2_At3g21610	10.000	atgggattcaaaaaggatgcttagc	agcctaacaccagtagcatcatacattac	55	750	*Dpn* II	2% agarose
C2_At5g06430	10.003	attgttatggctgatgcagagaatg	acgaagcaaggaacatactttatgtc	50	1000, 950	n/a	2% agarose
C2_At1g53000	10.008	agattctcggcaagcctatgatcc	aagctttgccctttcccatgttc	55	1800	*Dpn* II	2% agarose
T0787	10.009	aaccagtacagcgacaacttccga	caacattcaccaactcatactcgac	50	2300	*HaeIII*	2% agarose
TG303	10.011	cgtaaagggttgttcttgtgc	tgttttcgagtggggttcat	50	385	*Dde* I	2% agarose
TG303	10.011	cgtaaagggttgttcttgtgc	tgttttcgagtggggttcat	50	385	*Dpn* II	2% agarose
CT10670	10.013	unavailable[y]	unavailable[y]			n/a	Luminex
C2_At5g60990	10.014	tgatacactgaagcagcagtatcg	agccagaagacgagttgcatcac	55	1400	*Hinf* I	2% agarose
CT101051	10.030	unavailable[y]	unavailable[y]	55	191, 201	n/a	3% agarose
SSR318	10.031	gcagaggatattgcattcgc	caaaccgaactcatcaaggg	50	268, 270, 274, 278	n/a	6.5% polyacrylamide
SSR248	10.035	gcattcgctgtagctcgttt	gggagcttcatcatagtaacg	50	237, 239, 243, 247	n/a	6.5% polyacrylamide
LEVCOH15	10.037	gcaaccaccaatgttcattaca	aagctaaatctggcttgtggag	52	164, 170, 176, 178	n/a	4% agarose
CT104191	10.043	unavailable[y]	unavailable[y]	55	105, 124	n/a	3% agarose
C2_At1g67740	10.044	atgtgactccgcatttgcagctc	atctcatcttattaatctgattcaaagc	55	370	*Hinf* I	2% agarose
TG285	10.045	accaagcaaatgttgatgccctcg	ctggtctatgaatgctgtgacgct	55	1080	*Hpy* CH4III	2% agarose
CT100781	10.046	unavailable[y]	unavailable[y]	55	231, 238	n/a	4% agarose
CT10701	10.046	unavailable[y]	unavailable[y]			n/a	Luminex
C2_At3g58470	10.061	attgcttgtcccacactttatgc	tactgttcaaaccgtttgtcatactc	50	1000	*Tsp* 509I	2% agarose
TG233	10.086	catgccttttttcttgggatg	tggaaccccttttaactgtgc	55	490	*Hinc* II	2% agarose
TG403	10.095	tttgccttggttcccttatgcagc	gcagtgtgagccgggatatttgtt	55	953	*Dde* I	4% agarose
TG403	10.095	tttgccttggttcccttatgcagc	gcagtgtgagccgggatatttgtt	55	953	CelI	2% agarose
TG63	10.103	gcctttgaccctccctatatcaca	agcagaagcagatggttgagcagt	55	1250	*Hpy* 188I	2% agarose

Table B-2. Continued

Marker	Approximate position[z]	Forward primer	Reverse primer	Annealing temperature	Amplicon size(s)	Restriction enzyme	Detection
TG523	11.025	atttcctggattcgttttct	ctactacttcacttcctggtcat	56	350	CelI	2% agarose
T0408-1,2	11.026	tagacggtgctcatgtcgag	gttctcggcacccattctaa	55	700	*Mnl* I	2% agarose
CT20244I	11.034	unavailable[y]	unavailable[y]	45	277, 280	n/a	6.5% polyacrylamide
C2_At4g22260	11.037	tcctctaacggtctagagaaatggg	aggaactcttgcaattgtttccagaac	55	720	*Dde* I	2% agarose
CT182	11.038	gggagggaacaagttactcta	gccaacttcttaggccgtttc	55	544	*Rsa* I	2% agarose
cLEX-4-G10	11.044	atggtgcttgtgttgcttcg	ccttggttgcccgctgaa	55	384	*Rsa* I	2% agarose
CT10737I	11.053	unavailable[y]	unavailable[y]	55	163, 176	n/a	3.5% agarose
TG286-3	11.054	tggaaagcttcctccttcac	tcgactatgccatttgcttg	50	1200	*Hpy* CH4IV	2% agarose
CosOH57	11.054	tgcccaaaagcacagtacaa	cgcctcctatcttccaaactt	56	225	*Pfl* FI	2% agarose
TG400	11.057	tccaaatccaccacctatcc	agcattgctccctgctaaag	50	404	*Mnl* I	2% agarose
CT10615I	11.059	unavailable[y]	unavailable[y]	45	175, 182	n/a	6.5% acrylamide
TG384	11.059	tgatgatttgacctttgtccagg	accactatgttgctgatggca	52	1200	*Hha* I	2% agarose
SSR637	11.059	aatgtaacaacgtgtcatgattc	aagtcacaaactaagttaggg		156, 168, 181	n/a	6.5% polyacrylamide
cTOE-14-L16	11.060	gatgaagagaacagaactcctactc	ccaatctgaaaggatattccactg	58	800	CelI	2% agarose
TOM196	11.061	cctccaaatcccaaaactct	tgtttcatccactatcacga		207, 210	n/a	6.5% polyacrylamide
TOM144	11.062	ctgtttacttcaagaaggctg	actttaactttattattgcgacg	45	164, 170, 173, 182	n/a	6.5% polyacrylamide
CT20181	11.068	unavailable[y]	unavailable[y]			n/a	Luminex
C2_At3g54470	11.072	tcctgactttggttctaagcttagatcg	tcaaatattaagaagttgtgcttgtctgc	57	665	CelI	2% agarose
C2_At1g30825	11.072	atgtgaccgtcatatttcctatgag	agggggcattataagtccagcag	56	1050	*Bse* RI	2% agarose
cLET-24-J2	11.094	caaccatcctagcaatgaaatct	gaggcattcactctcttcgatac	55	394	*Hpy* CH4III	4% agarose
LEOH57	11.[x]	tggtcaacagatggtgaagaa	ggatcccatgccaatgaata	52	151	*Bst* UI	2% agarose

Table B-2. Continued

Marker	Approximate position[z]	Forward primer	Reverse primer	Annealing temperature	Amplicon size(s)	Restriction enzyme	Detection
CT10925I	12.001	unavailable[y]	unavailable[y]	55	156, 163	n/a	4% agarose
CT10953I	12.029	unavailable[y]	unavailable[y]	55	219, 231	n/a	4% agarose
TG360	12.032	ccccagaacacctctccata	tttcccgattttgttcctga	55	1050	*Apo* I	2% agarose
CT100	12.036	taacttggggcgaaggac	cagcagaaaagccttgagg	56	1000	*Rsa* I	2% agarose
SSR20	12.037	gaggacgacaacaacaacga	gacatgccacttagatccacaa	50	185+190+194	n/a	4% agarose
C2_At4g16710	12.040	agtttttgtgaccgtggggacaac	tgaacctgcgtggctgataacaag	55	1500	*Taq* I	2% agarose
CT99	12.045	gtcccggtgacatacttactg	agattctgtgttggaggtgagt	50	850	*Hpy* CH4III	4% agarose
TG565	12.048	ttcacagctggtgtctttcg	ttgcaagtggatgacagagg	55	1300	*Alu* I	2% agarose
T1736	12.052	attctcgatcaacggaccac	acactgagcaatgcgaatca	48	1200, 450, 300	n/a	2% agarose
C2_At5g42740	12.055	agcaccatttgagaaaaatatacctg	atccaaggaatgaaacattccacac	55	1070	*Dde* I	2% agarose
C2_At4g18593	12.059	aggtgattgttataatcgtggagaaag	ttcacaatgcgcacataaaagcttg	55	900	*Sau* 96I	2% agarose
LEOH301	12.063	tgctgttttgtttggctcac	tgttcatatctttgatggcatgt	52	164,185	n/a	4% agarose
CosOH1	12.070	tgcatacacttggtcatgacttc	ggctatagcatgcgttggtt	54	506	*Tsp* RI	2% agarose
LEOH275	12.071	tcctctgaaaacaacttcacga	agtgtgagcctcaaattcca	52	144	*Mse* I	2% agarose
CT10329I	12.078	unavailable[y]	unavailable[y]	55	471, 517	n/a	3% agarose
CT10778	12.079	unavailable[y]	unavailable[y]			n/a	Luminex
CT10796I	12.080	unavailable[y]	unavailable[y]	55	170, 180	n/a	4% agarose
LEOH197	12.081	tctgatgttggtagagccattg	tgatcataatgtgacgaatcgaa	52	143, 152	n/a	6.5% polyacrylamide
PtiB	12.[x]	gcccctgatatggcagcacgtc	caaggcagcaactgcagccatc	56	700	*Mnl* I	2% agarose

Table B-2. Continued

Marker	Approximate position[z]	Forward primer	Reverse primer	Annealing temperature	Amplicon size(s)	Restriction enzyme	Detection
CT20156I	not mapped	unavailable[y]	unavailable[y]	45	132, 135	n/a	6.5% polyacrylamide
CT10042I	not mapped	unavailable[y]	unavailable[y]	55	107, 131	n/a	3% agarose
CT10100I	not mapped	unavailable[y]	unavailable[y]	55	300, 320	n/a	3% agarose
CT20074I	not mapped	unavailable[y]	unavailable[y]	55	152, 159	n/a	4% agarose
CT20202I	not mapped	unavailable[y]	unavailable[y]	55	198, 202	n/a	CelI
SSR71	not mapped	aaatggcatggagaatggaa	catccactgagagcccaaag	50	238, 240, 242	n/a	6.5% polyacrylamide
C2_At3g54360	not mapped	agctttcctggttcaacaagcc	aactgctccgagctgtgagcac	53	350, 270, 85	n/a	2% agarose
C2_At2g25950	not mapped	tggtggtgctgatggaacaagtcc	tcgcttgcatagattgagcatctg	50	375, 700	n/a	2% agarose
CT10793	not mapped	unavailable[y]	unavailable[y]			n/a	Luminex
CT10483	not mapped	unavailable[y]	unavailable[y]			n/a	Luminex
CT10218	not mapped	unavailable[y]	unavailable[y]			n/a	Luminex
CT10432	not mapped	unavailable[y]	unavailable[y]			n/a	Luminex
CT10012	not mapped	unavailable[y]	unavailable[y]			n/a	Luminex
CT10050	not mapped	unavailable[y]	unavailable[y]			n/a	Luminex
CT10943	not mapped	unavailable[y]	unavailable[y]			n/a	Luminex
CT10425	not mapped	unavailable[y]	unavailable[y]			n/a	Luminex
CT20001	not mapped	unavailable[y]	unavailable[y]			n/a	Luminex
CT10205	not mapped	unavailable[y]	unavailable[y]			n/a	Luminex

[z] Number represents chromosome and map position (in cM).

[y] For primer sequences, contact Dr. David Francis, OSU, Wooster, OH

[x] Precise map position has not been determined.

Table B-3. Markers determined by restriction digestions to be non-polymorphic among genotypes resistant or susceptible to bacterial spot.

Marker	Approximate position[z]	Reference source for primers
TG236	1.023	SGN
C2_At3g06580	1.026	SGN
HBa0003D15.1-1	1.029	Present
C2_At1g14310	1.031	SGN
C2_At5g49480	1.032	SGN
C2_At3g62010	1.040	SGN
C2_At4g30890	1.040	SGN
C2_At4g01880	1.041	SGN
C2_At2g47210	1.043	SGN
TG59	1.097	Aliya Momotaz, personal comm.
C2_At4g29120	1.112	SGN
C2_At1g10240	1.128	SGN
C2_At4g22200	1.132	SGN
C2_At5g64350	1.137	SGN
HBa0010D01	1.140	Present
T1494	2.080	Aliya Momotaz, personal comm.
C2_At4g38630	2.084	SGN
HBa0009K06.1	2.084	Present
C2_At5g66530	2.088	SGN
HBa0104A12	2.088	Present
TG167	2.088	Aliya Momotaz, personal comm.
TG151	2.093	SGN
TG599	3.085	Astua-Monge et al, 2000
HBa0082F22	3.087	Present
C2_At5g23060	3.103	SGN
C2_At5g07910	3.112	SGN
C2_At1g74520	3.113	SGN
T0707	4.000	SGN
HBa0079G02	4.012	Present
C2_At1g68100	4.013	SGN
U234506	4.046	SGN
C2_At2g20390	4.028	SGN
HBa0029F16.1	4.034	Present
C2_At4g25650	4.037	SGN
T1405	4.077	SGN
HBa0255I02.1	4.080	Present
C2_At4g09010	4.083	SGN
HBa0053M02.1	4.088	Present
U214856	4.094	SGN
C2_At5g14320	5.010	SGN
cLEX-13-I3	5.013	SGN
U227536	5.015	SGN
C2_At1g07040	5.017	SGN
CD64	5.027	Aliya Momotaz, personal comm.
CT93	5.037	Aliya Momotaz, personal comm.
C2_At2g01110	5.037	SGN
TG626	5.043	Present
C2_At3g55120	5.044	SGN
C2_At2g03510	5.055	SGN
C2_At1g26520	5.056	SGN
C2_At1g14000	5.059	SGN

Table B-3. Continued. Non-polymorphic markers.

Marker	Approximate position[z]	Reference source for primers
U221402	5.082	SGN
C2_At3g17210	5.100	SGN
C2_At5g49510	5.101	SGN
C2_At3g55360	5.108	SGN
C2_At2g39690	6.005	SGN
C2_At4g01900	6.008	SGN
T0507	6.025	SGN
C2_At4g27700	6.027	SGN
C2_At5g56940	7.011	SGN
HBa0179K09.1	7.012	Present
C2_At1g19140	7.024	SGN
C2_At3g13050	7.028	SGN
U242881	7.030	SGN
TG252	7.031	SGN
C2_At5g14520	7.038	SGN
C2_At2g04780	7.041	SGN
C2_At2g42810	7.045	SGN
CT52	7.047	Present
U216327	7.052	SGN
C2_At1g78620	7.057	SGN
CT54	7.059	Aliya Momotaz, personal comm.
C2_At3g14910	7.061	SGN
HBa0025I17-1	8.004	Present
HBa0025I17-2	8.004	Present
C2_At4g32280	8.016	SGN
C2_At5g11490	8.032	SGN
C2_At4g31115	8.036	SGN
C2_At3g04600	8.037	SGN
C2_At5g11480	8.039	SGN
HBa0076J13.1	8.047	Present
TG505	8.053	Present
C2_At5g47010	8.056	SGN
TG510	8.057	SGN
C2_At4g12230	8.064	SGN
TG18	9.014	SGN
C2_At2g36930	9.015	SGN
C2_At3g09925	9.025	SGN
CT220	9.112	Aliya Momotaz, personal comm.
T1065	9.116	SGN
CT10082I	10.000	Matt Robbins, personal comm.
C2_At3g54360	10.056	SGN
LEOH336	10.083	Tomatomap.net
I2	11.[y]	Tomatomap.net
LEOH19	12.043	Tomatomap.net
LEOH38.2	ɒt mapped	Dave Francis, personal comm.
CT10482	ɒt mapped	Sung-Chur Sim, personal comm.

[z] Number represents chromosome and position (in cM).

[y] Precise map position has not been determined.

APPENDIX C
DNA SOURCES FOR SELECTIVE GENOTYPING OF RESISTANT AND SUSCEPTIBLE SELECTIONS

Table C-1. DNA sources for selective genotyping of resistant and susceptible selections from Fla. 8233, Fla. 8517 and Fla. 8326 families.

	DNA source		
Selection	Fla. 8233 Family	Fla. 8517 Family	Fla. 8326 Family
Resistant			
1	Su07E748-SBK(4,5)[z]-BK	Su07E763-SBK(5,6,9,10,15,17)-BK	Su07E736-SBK(9,15)-BK
2	Su07E749-SBK(6,10,13,16,17)-BK	Su07E770-SBK(7,11,13)-BK	Su07E737-SBK(1,11,12,14,17)-BK
3	Su07E757-SBK(4,7,13)-BK	Su07E773-SBK(2,8,11,13,15)-BK	Su07E738-SBK(2,8,12)-BK
4	Su07E742-SBK(1,8,9,10)-BK	Su07E776-SBK(2,6,8)-BK	Su07E860-SBK(1,8,9)-BK
5	Su07E746-SBK(9,15,16)-BK	Su07E714-27-BK	Su07E721-12-BK
6	Su07E747-2-BK	Su07E714-152-BK	Su07E721-111-BK
7	Su07E707-20-BK	Su07E714-153-BK	Su07E732-SBK(4,5,17)-BK
8	Su07E707-22-BK	Su07E714-164-BK	n/a
9	Su07E707-27-BK	Su07E714-191-BK	n/a
10	Su07E707-31-BK	n/a	n/a
11	Su07E707-191-BK	n/a	n/a
Susceptible			
1	Su07E861-SBK(10,15)-BK	Su07E862-SBK(4,5,9,14)-BK	Su07E733-SBK(1,2,4)
2	Su07E743-SBK(1,9)-BK	Su07E864-SBK(5,10,11)-BK	Su07E735-SBK(4,8,11)-BK
3	Su07E707-9-BK	Su07E764-12-BK	Su07E739-SBK(5,10,11,13)-BK
4	Su07E707-65-BK	Su07E768-SBK(4,13)-BK	Su07E734-SBK(11,13)-BK
5	Su07E707-182-BK	Su07E714-32-BK	Su07E721-36-BK
6	n/a	Su07E714-88-BK	Su07E721-93-BK
7	n/a	Su07E714-156-BK	Su07E721-170-BK
8	n/a	Su07E714-188-BK	Su07E721-174-BK

[z] Numbers in parentheses refer to actual plants used in special bulks (SBK).

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BIOGRAPHICAL SKETCH

Samuel Forrest Hutton was born on November 14, 1977 in Greenwood, MS. He grew up with an older and two younger sisters in Tchula, MS, where his father farmed cotton, rice, soybean and corn. He worked for his father each summer until graduating from Cruger-Tchula Academy in 1996. Sam then attended Delta State University for one year before transferring to Mississippi State University, where he received his B.S. in agronomy in December 2000. He began graduate school at the University of Minnesota in August of the following year and married Emily D. Jones one year later. The two of them remained in MN until June 2004 when Sam graduated with a M.S. in soybean breeding.

In August 2004, Sam began his doctorate in tomato breeding at the University of Florida under Dr. J.W. Scott. He lived in Gainesville for nearly two years and then moved to Tampa for completion of his research at the GCREC. His first child, Anna Christine, was born in December, 2006, and his second arrived soon after his dissertation defense. Upon completion of his Ph.D., Sam continued working in J.W. Scott's tomato breeding lab as a post-doctoral researcher.