# GENETICS OF SOUTHERN BLIGHT RESISTANCE IN TOMATO (*Solanum lycopersicum* L.)

By

MEHUL SAMIR BHAKTA

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2010

To my family, for supporting and never losing faith in me; and to all my friends along the way for without all of you this would not have been possible.

ACKNOWLEDGMENTS

I express my deep appreciation to Dr. Jeremy Edwards, esteemed chair of my advisory committee, for his persistent support, encouragement and scientific guidance during my association with him. This work would not be possible without his help and guidance. I would specially like to thank Dr. Xin Zhao and Dr. Gary Vallad the other members of my advisory committee for their immense support and creative suggestions.

I would also like to express a deep sense of gratitude and sincere thanks to Dr. Jay Scott for introducing me to Dr. Jeremy Edwards and also for his kind help and valuable suggestions, to Dr. Jeffrey A. Rollins for his assistance and suggestion for my project in Gainesville. I am also very grateful to Dr. Samuel Hutton for his guidance and support during this entire project and also for contributing valuable marker information required for this project.

I pay special thanks to my lab members Timothy Davis, Ragy Ibrahem, Cathy Provenzano, Jose Diaz for their support in lab, greenhouse and field work. I would also like to thank my fellow graduate student Xie Chenzhao for her support with my inoculation work.

I am deeply grateful to all my friends who filled my life with joy and happiness and made my stay at GCREC a memorable event of my life. I thank my family for believing in me and for their support in pursuing this degree.

Above all, I thank God for making me capable in achieving this goal.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

GENETICS OF SOUTHERN BLIGHT RESISTANCE IN TOMATO
(*Solanum lycopersicum* L.)

By

Mehul S. Bhakta

August 2010

Chair: Jeremy D. Edwards
Major: Horticultural Sciences

Southern blight caused by *Sclerotium rolfsii* Sacc. is a soil-borne fungal disease of a wide range of plant species occurring throughout tropical and sub-tropical regions. This study aimed to evaluate the level of southern blight resistance provided by various genetic sources in tomato and also to determine if isolates of *S. rolfsii* differed in their virulence against tomato, so as to enable us to identify the most effective sources of resistance which could be used to identify molecular markers closely linked to loci conferring resistance to southern blight. In order to check the resistant sources and virulence level in different isolates, two different resistant sources (PI 126932 and 5913M) and a susceptible source (Fla. 7776) were inoculated with three different isolates of *S. rolfsii*. The plants were inoculated at an age of eight weeks by *S. rolfsii* grown on rye seeds. Disease severity was estimated by scoring individual plants on a visual scale of 0-4 with increasing severity, and overall survival was recorded. For identifying linked markers, a mapping population was generated from a cross between Fla. 7776 and PI 126932. The parental lines, $F_1$ and $F_2$ individuals, $F_3$ families and $BC_1$ individuals were assayed for southern blight resistance. The selective genotyping

method was used to screen $F_2$ population with 102 co-dominant molecular markers distributed throughout the genome. Significant markers were confirmed with additional $F_2$ individuals as well as with $F_3$ and $BC_1$ generations. Results indicated that PI 126932 was resistant against all three isolates while 5913M against only two isolates (WM 609 and DF/LA-SR1). Also, differences in disease severity among isolates were observed in line 5913M and PI 126932. This indicated that southern blight resistance in tomato could depend on the interaction between the tomato genotype and southern blight strain. The percent of surviving individuals increased from 10% in the susceptible parent (Fla. 7776) to 90% in the resistant parent (PI 126932) suggesting incomplete penetrance. Two loci, L1 on chromosome 10 and L2 on chromosome 11, were associated with the resistance to southern blight. Results indicated overdominant and epistatic effects at both loci. The identification of favorable alleles in both parents explained recovery of transgressive segregants among progeny derived from the cross between Fla. 7776 and PI 126932. Apart from such epistatic genetic interactions, the locus L1 from PI 126932 provided sufficient resistance under greenhouse conditions as a dominant trait. Results from this study suggest that the L1 locus was an ideal source of southern blight resistance that could be introgressed into elite tomato lines through marker assisted backcrossing.

# CHAPTER 1
# INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and consumed vegetable crops in the world. World production of tomato in 2008 reached 129.64 million metric tons, out of which 12.57 million metric tons of tomatoes were produced by the United States of America (FAOSTAT). Florida is the largest producer of fresh market tomatoes in the United States, producing 474.36 thousand metric tons in 2008 and 557.82 thousand metric tons in 2009 (USDA). The tomato industry in Florida is important to the state economy, contributing a value of more than $997 million, worth $299 million in labor income (VanSickle and Hodges, 2008). However, there are many challenges being faced by this industry, such as rising production costs, reduction in farming area due to urbanization, increases in disease occurrence due to regulatory phase out of chemicals, global climatic changes etc. Southern blight of tomato is one of the soil-borne diseases which could become a major problem in near future due to the phase out of the soil fumigant Methyl Bromide (an ozone depletor) (Gordon and Taylor, 1941).

*Sclerotium rolfsii* Sacc. (teleomorph *Athelia rolfsii*; *Corticiaeae*, *Basidiomycota*), a soil-borne fungus, is responsible for significant economic losses on a wide range of agronomic host plants. The most common hosts are the legumes, crucifers, and cucurbits. On tomato, the disease is referred to as southern blight (synonyms: stem rot, southern root rot, sclerotium blight) and is associated with warm, noncalcareous acid soil (Sherf and MacNab, 1986). The fungus is common in tropical and sub-tropical parts of the world and infects more than 500 species of plants in over 100 families and is a problem in many southeast parts of the United States (Aycock, 1966). It appears that *S.*

*rolfsii* is mostly confined to areas where average winter temperatures are not cold enough to kill mycelia and sclerotia in the soil. Trees are found to be more resistant to this disease once they have passed their seedling stage (Freire *et al.*, 2002; Naqvi, 2004).

The first documented report of southern blight was reported by Peter Henry Rolfs' in association with tomato blight in Florida in 1892 (Garren, 1959). The organism was later named as *S. rolfsii* and was placed in the phylum *Fungi Imperfecti* (Deuteromycota) by Saccardo in 1911. Aycock, 1966 in his work cited many people who worked with a perfect stage developed in cultures of *S. rolfsii* from 1930 to 1941. The Basidiomycete teleomorph of *S. rolfsii* was first reported in 1931 (Curzi, 1931). Curzi named it *Corticium rolfsii* (Sacc.) Curzi since there was no known basidiomycete corresponding to the sexual stage of this fungus. In 1934 the perfect stage was first reported in the United States (Barret, 1934), followed by Mundkur (1934) on onion in India. Goto in 1935 obtained both typical and atypical isolates of *S. rolfsii*. Milthorpe in 1941 also observed differences in mycelia characters and in the production of sclerotia among seven single basidiospore isolates. All agreed that the perfect stage of *Corticium* sp;. *Corticium centrifugum* (Lév.) Bres. was most similar to the characteristics of the new fungus, in color and thickness for basidium structure. As a result, the name *Corticium rolfsii* (Sacc.) Curzi, proposed by Curzi was generally adopted. However, a proposal that the fungus should be placed in the genus *Pellicularia* and named *Pellicularia rolfsii* (Sacc.) West was proposed by West in 1947 giving the reason that it conformed morphologically with characteristics of the subdivision of *Corticium* (West, 1961).

Extreme morphological and less marked physiological differences have been known among several *S. rolfsii* strains. It has been shown that isolates from a given geographical area may be relatively uniform, and verifiable mutants are less frequent. Even so, single-basidiospore cultures from one of these isolates frequently show the extreme variation of the species. In a study of the perfect stage of *S. rolfsii*, Lyle obtained 306 monobasidiospore isolates from two original isolates which produced hymenia in culture (Lyle, 1953). A marked difference was noticed by Lyle in the growth type, amount of vegetative growth, sclerotial characters, mutual effects of sclerotial and asclerotial isolates, aversion and hymenial formation. These differences lead Lyle to conclude that the fungus can be homothallic. Goto (Aycock, 1966) reported earlier that on pairing of larger numbers of monobasidiospores, several strains of varying characteristics including parental strains were obtained indicating that the basidiospore cultures were usually heterothallic. The uniformity of isolates from an area may be an expression of dominance in the dikaryon, where as the great diversity reported in vitro could be an expression of recombination and sorting out of nuclei in the sexual stage (Lyle, 1953).

*S. rolfsii* isolates were reported to be showing distinction not only in their morphology but also in their pathological behavior (Harlton *et al.*, 1995; Sharma *et al.*, 2002; Shukla and Pandey, 2007; Shukla, 2008). Edson in 1923 used two isolates of *S. rolfsii* in their study and reported that they differed pathogenically as well as morphologically. Shukla and Pandey in 2008 tested 10 isolates and observed four distinct pathogenicity reactions against *Parthenium hysterophorus* L. They noted that depending upon the isolate, the disease incidence in a given individual ranged from 30

to 80 percent. Finding of Shukla and Pandey supported the finding of Flores-Moctezume *et al.* (2006) who also reported four levels of pathogenicity in two of the isolates they tested against different species like *Ricinus communis, Sesamum indicum, Tagetes erecta* etc.

*Sclerotium rolfsii* is known to infect a diverse array of plants. Rolfs (West, 1961) mentioned about 15 host plants that he observed which included weeds and garden plants. Some of the hosts reported to be affected by this fungus in the U.S. includes *Arachis hypogaea, Beta vulgaris, Brassica oleracea, Capsicum annum, Cucurbita* spp. *Citrusllus vulgaris, Daphne* spp, *Ficus carica, Gossyium hirsutum, Phaseolus vulgaris, Solanum tuberosum, Solanum melongena, Solanum lycopersicum, Pensiemon* spp, *Phlox sublata* etc (Taurbenhaus, 1919). Webber, in 1931 published a list of 189 species of plants susceptible to southern blight which included 8 monocot and 42 dicot families. Many more hosts susceptible to this fungus have been reported later in the publications from many parts of the tropic and sub-tropic regions around the world. The list included host plants with high economic value along with ornamentals, forest species and weeds (West, 1961). Recently reported hosts include *Phaius flavus* (Bl.) Lindl. and *Paphioedilum venustum* (Wall.) Pfitz.ex Stein. (Bag, 2003), *Dioscorea alata* (Jeeva, *et al*., 2005), *Swietenia macrophylla* and *Pterocarpus santalinus* (Sankaran, *et al*., 2007), *Ascocentrum* and *Ascocenda* orchids in Florida (Cating, *et al*., 2009), *Convolvulus cneorum* (Polizzi, *et al.*, 2010), *Musa* spp. (Thangavelu and Mustaffa, 2010).

In general, *S. rolfsii* is distributed in tropical and sub-tropical regions where high temperature prevails during the rainy season. Based on the occurrence record from publication, the geographical distribution of this fungus was estimated by West (1961).

He reported that *S. rolfsii* occurrence in the southern United States was found in Florida to California. South and Central American countries where reports of the presence of *S. rolfsii* have been obtained included Argentina, Brazil, Colombia, British Guiana, Trinidad, West Indies, Dominican Republic, Bermuda, Barbados, Jamaica, St. Vincent, Puerto Rico and Cuba. While publications from Italy, Germany and U.S.S.R reported presence in Europe. From Africa, reports have been obtained from Egypt, Tunis, Gold Coast, Sierra Leone, Gambia, Belgian Congo, Uganda, Southern Rhodesia, Nyasaland, Madagascar, and Union of South Africa. A number of articles have been published from countries like India, Iran, Japan, Malaya, China, Ceylon, Formosa in Asia reporting the occurrence of *S. rolfsii.* Occurrence reporting articles have also been published from Philippines, Java, Sumatra, Hawaii and Australia in the Pacific area (West, 1961).

The mycelium of *S. rolfsii* is able to grow in the temperature range of 8 to 40 $^{o}$C. However, the optimum range for growth is 30 to 35 $^{o}$C. Vegetative hyphae are killed by an exposure of 24 hrs to -2 $^{o}$C. Sclerotial formation was greatest in the temperature range of 30-35 $^{o}$C (Milthorpe, 1941), whereas the optimal temperature for the germination of sclerotia ranged from 24 to 36 $^{o}$C. Highest germination rate is obtained when the sclerotia are stored at relative humidity levels from 25% to 35% (Watkins, 1950; Punja, 1985). Povah (1927) showed that five years old sclerotia could be induced to produce mycelia growth.

Sclerotia exhibit two forms of germination: hyphal and eruptive (Punja and Grogan, 1982; Punja, 1985). In hyphal germination, an individual strand grows out from the sclerotium surface. However the growth is not extensive in absence of an external nutrient source. In the case of eruptive germination, an aggregate of mycelium emerges

from the sclerotial rind. Eruptive germination is induced by drying the sclerotia (Smith, 1972; Punja and Grogan, 1982) or by exposing them to volatile compounds, mainly alcohols and aldehydes (Punja, 1985).

Disease symptoms on the plant are generally accelerated by favorable temperature. The period of incubation is approximately 2-4 days for tomato plants. An early symptom of infection is manifested by a deep brown lesion on the stem at the soil line. During infection mats of mycelium develop around the lesion on the stem base of tomato seedlings. These mats are attached to the stem by the hyphae which are appressed to the host epidermal cells. Death of the underlying parenchyma cells occurs to a depth of two to four layers before they are penetrated by hyphae (Aycock, 1966). Later the foliage droops, loses its green color and the plant never revives.

Oxalic acid is considered a pathogenicity factor in *Sclerotium rolfsii*. On direct application of oxalic acid on stem or either leaf tissue, the resulting injury and wilting symptoms observed were found to be similar to as caused by *S. rolfsii* (Malcolm, *et al*., 2005). Bateman and Beer (1965) concluded that wilting is induced as a consequence of acidifying the host tissue due to oxalic acid. Prominent activities of polygalacturonase and cellulose were detected in infected tissue (Bateman and Beer, 1965; Bateman, 1972). A number of pathways have been proposed by which oxalic acid could aid infection, such as acidification to facilitate cell wall degrading enzyme activity, through tissue damage by pH, or by $Ca^{++}$ ions sequestration from the cell walls to form calcium oxalate (Dutton and Evans, 1996; Malcolm, *et al*., 2005). Amadioha (1993) reported that oxalic acid could work synergistically with polygalacturonase during the initiation of infections. Polygalacturonase was found to be hydrolyzing calcium pectates only when

oxalate ions were present, which indicated that polygalacturonase and oxalic acid produced by *S. rolfsii* acted together while disrupting the host cell walls during infection (Bateman and Beer, 1955).

Earlier work showed that sclerotia can remain viable up to 5 years (Povah, 1927; Nisikado *et al*., 1938). The extensive host range and prolific growth of *S. rolfsii* and its ability to produce large number of sclerotia that may persist in soil for several years increases the difficulty to control the resulting diseases.

Alteration of soil pH was one of the earliest strategies for controlling the diseases caused by *S. rolfsii*. Higgins (1923) and Rosen (1929) both suggested applying lime to increase the soil pH to around 8 for disease control. Later, Higgins (1934) found liming to be impractical due to the cost of treating entire fields and difficulty of maintaining pH around 8. Reducing the soil pH to around 2.4 was ineffective at controlling disease (Aycock, 1966).

Host nutrition was reported to influence disease resistance in a number of instances. Leach and Davey (1942) found that calcium nitrate effectively reduced severity of Southern blight. It was postulated that N fertilizers may induce anatomical or physiological resistance in the host (Mohr, 1955). Hudgins (1952) conducted an experiment that varied the composition of N, P and K in peanut and also found that higher levels of N decreased disease severity.

Mohr and Watkins (1959) reported that calcium nitrate depressed disease expression. They also reported that the disease was more severe on sandy soil as compared to the clay soil. On further analysis of the soil they found that the clay soil had 10 times more calcium, and noted that application of calcium nitrate to a susceptible

variety enable it to survive a week longer as compared to the plants fertilized with ammonium sulfate and sodium nitrate. They concluded that nitrogen was probably not the principal element associated with resistance to *S. rolfsii*; rather a resistant variety may be more efficient in absorbing and utilizing calcium.

Grafting resistant rootstocks to susceptible scions may be another effective strategy to control diseases caused by soil-borne pathogens. Several rootstocks like 'Big Power', 'Beaufort', and 'Maxifort' were resistant to southern blight and found to reduce disease severity when utilized for tomato production (Rivard *et al*., 2009). However, grafting increases the crop production time and also labor cost, which limit its widespread adoption. Control of southern blight by the use of bio-control agents has also been proposed. Bio-control agents like *Trichoderma koningii* protected tomato seedlings against *S. rolfsii.* (Latunde-dada, 1993). Ganesan *et al.* (2007) reported that the combined application of selected antagonistic Rhizobium isolates and the bio-control agent *Trichoderma harzianum* conferred significant protection to *Arachis hypogaea* L. against *S. rolfsii* and increased plant growth. It reduced the disease incidence by 57% as compared to the control. Although considerable control has been obtained by bio-control agents they have not been accepted widely because of the limited performance *vis-à-vis* chemical fungicides and fumigants. In addition, microorganisms used for biological control can have significant, measurable effects, both direct and indirect, on non-target organisms. These effects include displacement of non-targeted soil microorganisms, allergenicity to humans or animals and toxigenicity or pathogenicity to undesired organisms (Brimner & Boland, 2004; Cook, et at, 1996). Boyel (1952) and Garren (1959d) proposed that deep burial of organic matter to reduce

the occurrence of southern blight. However, Young (1954) stated that deep burial will reduce crop yields for at least few years, since infertile soil is brought to the soil surface.

Because of the low effectiveness of these methods, the management of southern blight has relied heavily on the application of chemicals and crop rotations (Leeper *et al.*, 1992). Although crop rotation has been suggested as a control for southern blight by many, the extensive host range and survival period of sclerotia in soil has limited this cultural practice.

Several fumigants have been found to be effective for the management of *S. rolfsii*. Chloropicrin at a rate of 100 ppm was found to be highly effective than most of the chemicals tried (Davey and Leach, 1941). Pentachloronitrobenzene (PCNB) was also found to be an effective fungicide in curbing the southern blight. Csinos, *et al.* (1983) found that PCNB reduced southern blight incidence by 50% in peanut. However, in April 1993 PCNB was declared a hazardous air pollutant in the U.S. (Howard, 1991), also the use of Chloropicrin was restricted by the US government in June 2003 (EPA, 2007), although such restriction was withdrawn latter. Another fumigant, Methyl bromide (MeBr) gave adequate control of *S. rolfsii*, and this fumigant has been widely used throughout the globe to treat soil in infested beds (Aycock, 1966; Jenkins and Averre, 1986; Brown *et al.*, 1989). MeBr is an effective fungicide, herbicide, nematicide and insecticide and has been used commercially in United States for soil fumigation (Ragsdale and Wheeler, 1995).

MeBr has been used by Florida's tomato and pepper growers well over 40 years, which has unfortunately limited the development of alternative multiple pest control tactics for many of the soil-borne pests of these crops (Chellemi, 1998). However, the

provisions of the Montreal Protocol will eventually lead to a complete phase out of MeBr use in crop production excluding the critical use exemption. If no alternatives are available then the economics of producing certain horticultural crops in states like Florida, North Carolina, California and other southern states will be greatly affected by the ban imposed on the use of MeBr (USDA, 1993; Spreen, *et al.*, 1995; CDFA, 1996). Due to the recent MeBr phase-out, Southern blight as well as other soil-borne pathogens which are currently a minor problem in Florida have a high potential of becoming major production issues. This threat has created the need to develop alternative strategies for controlling southern blight in tomato.

Even if a chemical replacement for MeBr is found, it is possible that it might not be economically feasible, lack the same efficacy against soil-borne pathogens, or could face a similar phase-out process in future. Thus, heritable resistance offers a particularly desirable long term solution to the problem of controlling southern blight. Early breeding efforts to develop resistant varieties confronted problems related to genetic variation in the pathogen, environmental effects on pathogenicity and expression of resistance in host (Mohr, 1955). Inheritance studies have shown that resistance in many species is monofactorial as compared to other species where resistance was shown to behave as a quantitative character with polygenic inheritance (Mohr, 1955). Such complexity underlies the challenge faced by plant breeders to develop resistant varieties that are horticulturally acceptable. For tomato, the first challenge to developing southern blight resistant varieties is to understand the nature of resistance along with its inheritance.

Numerous studies showed that tomato species *S. lycopersicum* carries little or no resistance against southern blight, although some tests found some variation in the degree of susceptibility (Fajardo and Mendoza, 1935; Mohr, 1955; Aycock, 1966). Mohr *et al.* (1947) found resistance to southern blight in a single plant introduction line of *S. pimpinellifolium* (PI 126932) obtained from Peru. In field screenings (Mohr, 1955), none of these plants died from southern blight though they were grown in a heavily infested area, while disease incidence was high among susceptible varieties.

Resistance in *S. pimpinellifolium* was proposed to be associated with the development of a ring of heavily suberized phellem cells that form a protective barrier around the stem when the plants were 6 to 9 weeks old (Mohr 1955). Southern blight infection can greatly increases on these plants if the phellem ring is somehow damaged (Jenkins and Averre, 1986). Such a lignified stem is not observed in *S. lycopersicum*. PI 126932 was not found to be resistant until they were about 6 weeks old. Mohr suggested that the resistance could be associated with a barrier to fungal penetration present in the outer stem tissue of mature plants but absent in young seedlings. Beside PI 126932, six other advanced breeding lines (5635M, 5707M, 5719M, 5737M, 5876M and 5913M) were identified to be resistant to southern blight in a breeding program intended to develop heat-tolerant processing-type tomato cultivars. These advanced breeding lines have been released as a source of southern blight resistance from Texas A&M University (Leeper, *et al.*, 1992)

Mohr (1955) reported that southern blight resistance in *S. pimpinellifolium* is inherited as a dominant, monogenic trait, but suggested that further screening was required to do an accurate analysis of the mode of inheritance. If resistance is inherited

as a single dominant gene, then the development of resistant varieties can be accelerated through the identification of molecular markers linked to the gene, allowing for marker assisted selection. Not only will the linked markers help in the rapid development of resistant lines, but they also would be of great use in pyramiding resistance alleles at multiple genes. The purpose of this study was to identify the locus imparting resistance to southern blight in *S. pimpinellifolium* to develop resistant lines of cultivated tomatoes. Specific objectives are 1) to develop reliable greenhouse methods to assay for southern blight resistance in tomato, 2) to confirm sources of genetic resistance in tomato, and 3) to map the genomic positions of the loci conferring resistance.

# CHAPTER 2
# SOURCES OF GENETIC RESISTANCE IN TOMATO TO SOUTHERN BLIGHT UNDER FLORIDA CONDITION

## Introduction

Southern blight of tomato (*Solanum lycopersicum* L.) caused by *Sclerotium rolfsii* Sacc. is a soil-borne disease that has the potential of becoming a major disease in Florida following the phase-out of Methyl Bromide (MeBr). This disease can cause major losses to tomato production in the southern United States. Georgia alone suffered a loss of $10.4 million in peanut crops due to southern blight with an additional $19.2 million spent on its control in 2004 (University of Georgia, 2005).

Infection is promoted by dense planting, high soil moisture and frequent irrigation (Aycock, 1966; Sconyers *et al.*, 2005). In tomato this fungus can infect all portions of the plant touching the soil, and sclerotia provide the primary inoculum for epidemics (Ristaino *et al.*, 1991; Liua *et al.*, 2008). Symptoms on tomato initiate with the decay of the cortex at the base of the stem several centimeters above and below the soil surface (Aycock, 1966), followed by the growth of a white mat of mycelia on the stem. Later sclerotia are produced, ranging from 1-2 mm in diameter, and tan to brown in color depending upon the strain. Infection causes partial or complete girdling of the stem near the soil line resulting in the damping-off of seedlings, while more mature plants develop a progressive wilt that begins with the lower leaves and eventually leads to plant death. Root infection can follow stem or crown invasion sometimes causing death of the tap root. Sclerotia can be found on fibrous roots about 2-4 inches deep (Aycock, 1966).Fruits touching infected stem or soil can also get infected. The infection site appears sunken at first and the epidermis may be ruptured by the time the lesion is 2 cm in diameter (Weber and Ramsay, 1926; Young, 1946; Aycock, 1966). Fruits can rot

in 3 to 4 days under ideal temperature and moisture (McColloch *et al.*, 1968; Jones *et al.*, 1991).

Several methods have been proposed to control this disease, but none has proven highly effective. The best possible control obtained was with the use of Methyl Bromide, a soil fumigant. This chemical was ranked as one of the five most used pesticides in the United States (UNEP, 1995). However, MeBr is photo-decomposed in the atmosphere by photons to release elemental bromine, which is highly destructive for the stratospheric ozone layer (Gordon and Taylor, 1941). Therefore, MeBr was designated as a class I ozone depleter and its use was phased out in United States by 2005 except for the critical use exemption under The Clean Air Act of 1990. Since no other chemical or cultural methods are available to control this disease as effectively as fumigation with MeBr, the best possible option is inherited host resistance.

Isolates of *S. rolfsii* have shown significant variations in their pathological behavior (Harlton *et al.*, 1995; Sharma *et al.*, 2002; Shukla and Pandey, 2007). In a study involving two isolates of *S. rolfsii* in 1923, Edson found that both differed not only morphologically but also with respect to their virulence towards potato. Flores-Moctezume *et al.* (2006) reported four levels of virulence reactions in two of the isolates they tested against different plant species like *Ricinus communis, Sesamum indicum, Tagetes erecta* etc. Their results were supported by Shukla and Pandey (2008) who tested 10 isolates and also observed four distinct pathogenicity reactions against *Parthenium hysterophorus*. They noted that depending upon the isolate, the disease incidence in a given individual plant ranged from 30 to 80 percent.

Along with differences in virulence among isolates of *S. rolfsii,* variation has been observed with respect to plant resistance and the mode of inheritance in different species. Mohr (1955) reported that the resistance in *S. pimpinellifolium* was controlled by a single, dominant gene. Whereas, in *Capsicum annuum* L. resistance was inherited as a single recessive gene (Fery and Dukes, 2005). In alfalfa, resistance against *S. rolfsii* was quantitative (Inami and Suzuki, 1981; Inami *et al.*, 1986; Pratt and Rowe, 2002).

The only documented sources of resistance to southern blight in tomato are selections from wild Peruvian accessions PI 126932 and PI 126432 of *S. pimpinellifolium* Mill and 6 breeding lines (5635M, 5707M, 5719M, 5737M, 5876M and 5913M) released from Texas A&M University, each containing *S. pimpinellifolium* in their pedigree (Leeper *et al.*, 1992). *S. pimpinellifolium* has proved to be a fertile source of resistant germplasm and hybridizes readily to *S. lycopersicum* (Muller, 1940). Introducing resistance to southern blight into cultivated tomato cultivars would provide a cost-effective and an environmentally safe method for managing this disease.

The objectives of this study were to assess the level of resistance provided by two genetic sources of resistance in tomato, and determine the inheritance of resistance. Results will help identify the most effective sources of resistance to incorporate into a breeding program and for genetic mapping, and will also establish disease assays for subsequent breeding and genetic studies.

## Materials and Methods

### Plant Materials

Fla. 7776 (*Solanum lycopersicum*), a southern blight susceptible cultivar was obtained from the University of Florida, Gulf Coast Research and Education Center

(GCREC), Balm, FL. While the resistant wild Peruvian accession of *S. pimpinellifolium* PI 126932 was obtained from the USDA, ARS, National Genetic Resources Program (Geneva, New York). An advanced breeding line, i.e., 5913M, was obtained from Texas A&M University. Seedlings of these lines were raised in 128- well styrofoam Speedling® trays (3.8 $cm^3$ cell size) in the greenhouse filled with sterilized peat-lite mix (Speedling Inc., Sun City, FL) in Fall 2008. The plants were fertilized with compound fertilizer 20-20-20 ($N - P_2O_5 - K_2O$) at an interval of 5 days. After 4 weeks, seedlings were transplanted into four inch diameter pots and maintained in the greenhouse for the duration of the experiment. Controlled pollination was carried out to hybridize Fla. 7776 (recipient parent) and PI 126932 in a greenhouse to generate $F_1$ progeny. $F_2$ seeds were produced and mass harvested by self-pollination of $F_1$ plants in the greenhouse. A $BC_1$ population was obtained by backcrossing $F_1$ with the recurrent parent Fla. 7776 in the greenhouse.

**Fungal Materials and Inoculum Preparation**

Three different isolates of *Sclerotium rolfsii* were used in this study. A strain of *S. rolfsii* (GCT-1) was isolated from an infected tomato plant at GCREC, Balm. Strain WM 609 was isolated from peanut in Georgia (Dr. Tim Brenneman, University of Georgia), and a strain recovered from sweet potato (DF/LA-SR1) was obtained from Louisiana (Louisiana State University).

Sclerotia from each isolates were submerged into 0.5 % sodium hypochlorite (NaOCl) solution for 5 min to sterilize the outer rind (Linderman and Gilbert, 1972). A single, surface sterilized sclerotium was transferred to a petri-dish containing potato dextrose agar (PDA), incubated at 25 $^oC$, to initiate cultures.

For inoculum preparation, approximately 200 g of rye seeds were washed with water in a 1 L Erlenmeyer flask and then immersed in 300 ml of water. The flask mouth was covered with 4 layers of cheese cloth followed by a layer of aluminum foil and was left overnight so that the seeds could absorb the water. The flasks containing rye seeds were autoclaved at 121 $^{o}$C for 15 min; this process was repeated for two consecutive days to ensure complete sterilization (Phatak and Bell, 1983). Sterilized rye seeds were then inoculated with approximately ten 9 mm PDA plugs containing both mycelium and mature brown sclerotia. The Erlenmeyer flask cultures were incubated at 25 $^{o}$C under 12 hr light and dark period for 3-4 weeks. The flasks were shaken thoroughly for the first seven days and then periodically as needed until the inoculum was used.

**Screening Study**

In order to find the inoculum load required for disease assays, five different inoculum treatments were tested using *S. rolfsii* strain GCT-1 in spring 2009. Eight-week- old plants of PI 126932 and Fla. 7776 in 4 inch wide pots were used in this study. Treatments consisted of 1) 1 g of inoculated rye seeds, 2) 1 g of inoculated rye seeds treated with 0.5% methanol spray, 3) 2 g of inoculated rye seeds, 4) 2 g of inoculated rye seeds treated with methanol and 5) 2 g non-inoculated rye seeds with methanol spray. Methanol was used to induce eruptive germination (Punja, 1985) in sclerotia in order to reduce the infection time. Each treatment was replicated 4 times with 6 plants in each replication and arranged in a randomized complete block design. For each of the treatments, the rye seeds were placed in contact with the host stem, on the soil surface and covered with 1cm of soil. The soil was kept constantly moist by frequent irrigation and the temperature was kept around 30 $^{o}$C. Plants were rated as dead or alive at the end of 4$^{th}$ week after inoculation.

**Isolate Study**

The trial was conducted using 44 plants each of PI 126932, Florida 7776 and 5913M which were sown in 128-well Styrofoam Speedling® trays (3.8 $cm^3$ cell size) containing peat-lite mix (Speedling Inc., Sun City, FL). The seedlings were grown to an age of 4 weeks, transplanted into 4 inch wide pots, and placed in the greenhouse. At 8 weeks after sowing, the plants were inoculated with isolate GCT-1, WM 609 and DF/LA-SR1 by dispersing 2 g of colonized rye seeds on the soil surface close to the stem base. The temperature in the greenhouse was maintained in the range of 27 to 32 $^oC$ and with soil moisture close to 50-70%. The trial was repeated once.

**Screening for Resistance with the GCT-1 Isolate**

Plants of PI 126932, Florida 7776, $F_1$, $F_2$ and $BC_1$ were evaluated for response to *S. rolfsii* in a greenhouse study arranged in randomized complete block design. Fifty seeds of each parent line, $F_1$, and $BC_1$ as well as 96 seeds of $F_2$ were sown in the speedling trays in fall 2009. Eight-week-old plants were grown and inoculated with *S. rolfsii* isolate GCT-1 as described earlier. Trial was also conducted to screen 4 week old seedlings, using 48 PI 126932 and Fla. 7776 seedlings inoculated in a growth room maintained at 28 $^oC$ with 80% relative humidity.

**Disease Assessment**

Seedlings grown in the greenhouse were examined every 2 days after inoculation for symptoms of wilting and for stem lesions. Disease severity was estimated by scoring individual plant on a visual scale of 0-4 for wilting with increasing severity: 0 = no wilting symptoms with initiation of stem lesion , 0.5 = initiation of wilting, 1 = 12.5% of total leaf area showing wilting symptoms, 1.5 = 12.5 - 25% wilted leaf area, 2 = 25% - 37.5% wilted leaf area, 2.5 = 37.5%- 50% wilted leaf area, 3 = 50%-75% wilting, 3.5 = >75%

wilting with lodged plant, 4 = Dead plant. Plants were rated 21 days after inoculation. Plants rated with a score of 2.5 or below were considered resistant plants as these plants were able to recover from the infection. While the plants with a score greater than 2.5 were considered to be susceptible since they were unable to recover and eventually died. A plant was considered to be an escape if no lesion or evidence of infection was noted on its stem. Plants rated as escapes were excluded from the study.

**Statistical Analysis**

For statistical analysis, data obtained from the isolate study were pooled across both trials. Disease severity based on genotypes and isolates were analyzed using a two-way analysis of variance of ranked data using the PROC Mixed procedure in SAS (version 9.2; SAS Institute Inc., Cary, North Carolina) to generate relative marginal effects (RME), and 95% confidence intervals as described by Brunner *et al*. (2002); Shah and Madden (2004); Vallad *et al*. (2006). Interactions between tomato genotype, *S. rolfsii* isolate and trials were also tested. A one-way analysis of variance was used to test for tomato genotype effect on disease severity scores caused by the isolate GCT-1. RME and 95% confidence intervals were generated as mentioned earlier for testing significant difference between disease score in tomato genotypes for GCT-1 isolate. Chi-square test was used to test for single gene model.

## Results

**Inoculation Procedure**

Only wilting scores were used to identify resistant plants as stem lesions were found on both Fla. 7776 and PI 126932. The most effective treatment found to distinguish the resistance and susceptible plants was 2 g of inoculated rye seed per plant (Fig 2-1), in which more than 90 percent of susceptible plants died, while all of the

resistant plants were able to survive (Table 2-1). Inoculation of 4 week old PI 126932 and Fla. 7776 resulted in the death of all plants.

**Genotype Effects**

Although PI 126932 exhibited severe stem lesions after inoculation with *S. rolfsii* isolate GCT-1, only mild to moderate wilting symptoms developed under the conditions of this study. Twenty days after inoculation, the susceptible inbred Fla.7776 developed severe stem lesion and wilting that led to plant collapse and death (Fig 2-1). No significant difference was obtained between the two trials (*P* value = 0.39). Also no interaction was found between tomato genotype x trial (*P* value = 0.74), isolate x trial (*P* value = 0.81), isolate x genotype x trial (*P* value = 0.82). Significant difference was obtained between all the three genotype tested (Table 2-2 and 2-3). PI 126932 and Fla.7776 differed significantly in their mean disease severity score (Table 2-4 and 2-5) following inoculation with *S. rolfsii* isolate GCT-1. However, the difference in mean disease severity between 5913M and Fla.7776 against GCT-1 isolate was not significant (Table 2-5). A statistical difference in disease severity between the two resistance sources was also observed (Table 2-5).

**Isolate Effects**

Disease severity scores were found to be affected by the isolate used for the inoculation. For the breeding line 5913M, a significant difference in the mean disease severity ranking was observed between peanut isolate (WM 609) and the tomato isolate (GCT-1) (Table 2-3). 5913M plants were killed by GCT-1, while they were able to survive against WM 609. For PI 126932, a significant difference was also observed in disease severity among various isolates (Table 2-3). For Fla. 7776, no significant isolate effect was observed. Such a variable response for 5913M and PI 126932 indicates that

the isolates differ in aggressiveness against specific tomato line. Significant interaction between tomato genotypes and isolates was also found (Table 2.3).

**Inheritance Patterns of Southern Blight Resistance**

Screening of the different generations of PI 126932 and Fla. 7776 with the GCT-1 isolate was carried out to understand the inheritance pattern (Fig 2-2). Out of the inoculated 50 plants of parent lines seven of the PI 126932 plants died, and about six of the Fla.7776 plants were able to survive although they showed severe stem lesions.

The $F_2$ progeny segregated in an approximate ratio of 1:3 [resistant (R): susceptible (S)] (Table 2-6, Fig 2-2). The segregation ratio in the $F_2$ population failed to fit the distribution expected for a single gene with dominant effect ($\chi^2 = 64$) based on Chi-square analysis; even when the expected segregation ratio was adjusted for the level of penetrance observed in both parents and heterozygous $F_1$ hybrids. Ratio also failed to fit the distribution expected for single recessive gene ($\chi^2 = 8.7$) when expected ratio was adjusted for penetrance level. Few of the surviving $F_2$ plants had a disease rating below 1 which was lower than mean disease score for the resistant parent PI 126932.

## Discussion

Using resistant varieties is an ideal approach for plant disease management. Resistant sources against southern blight have been identified in many hosts in a number of countries (Mohr, 1955; Sugha *et al.*, 1991; Besler *et al.*, 1997), but no stable resistance has been achieved due to the occurrence of aggressive isolates of *S. rolfsii* (Sharma and Jodha, 1984). We found that all three non-segregating populations i.e., the resistant parent PI 126932, the susceptible parent Fla. 7776 and the $F_1$ displayed a

range of susceptibility to *S. rolfsii* across individuals. This result was not unexpected based on earlier works. Aycock (1966) suggested that it is a characteristic that all plants even in a uniformly infested area do not become infected. Numerous published references to the irregular distribution of southern blight in the field were cited by Aycock (1966). Similar observations of irregularly distributed disease were also made by Fery and Dukes (2005) in pepper. They noted such variable reaction in most of the pepper cultigens evaluated in field trials which they conducted over multiple years. In their opinion such variable reactions to *S. rolfsii* observed in their parental and $F_1$ populations were not due to genetically heterogeneous plant material, but due to a complex environmental interaction. In this study complex environment interaction may have played an important role; however the possibility that the resistant plant introduction line was not completely fixed for resistance cannot be completely ruled out, and would require additional testing to verify.

5913M was found to be susceptible against GCT-1 but not against remaining isolates which suggests that 5913M is not a reliable resistance source for multiple isolates. Prior studies found that isolates from the same geographical location could be host specific or exhibit a narrower host range (Cilliers *et al.*, 2000). Punja and Sun (1997) compared 128 isolates of *S. rolfsii* from 36 host species and 23 geographic regions by means of random amplified polymorphic DNA (RAPD) polymerase chain reaction and found that many isolates from the same host belonged to the same mycelial compatibility group (MCG). The isolates used in the current study were found to fall into three different MCG groups (Xie Chenzhao and Gary Vallad, personal communication) and each was collected from different host species and different

geographical location (peanut-Georgia, tomato-Florida and sweet potato-Louisiana). Different MCG groups suggest that there is genetic variation among the isolates (Cilliers *et al.*, 2000) and could probably explain the variation in the disease severity score obtained in this study. *S. rolfsii* isolates from groundnut (peanut) fields in Texas were studied by Nalim, *et al.,*(1995) and based on DNA amplification pattern they found that the isolates which belonged to the same MCG were clonal, which was supported by the similarities in morphology and host specificity of the isolates they studied. This study included only three MCGs with one isolate in each group. To get a better understanding of difference in virulence among isolates, a thorough study needs to be carried out involving multiple MCGs with multiple isolates in each MCG, testing for pathogenicity on specific host plants.

Based upon the variability in response of 5913M to different isolates of *S. rolfsii*, it is possible that the resistance conferred by this advanced breeding line isolate-specific. Such a differential response to *S. rolfsii* isolates suggests that resistance breeding needs to be based on the isolates present in the targeted geographical location. On the contrary, PI126932 was found to be resistant against all three isolates tested. It is likely that the level of resistance found in PI 126932 is enough to overcome the attack of different isolates and this line could potentially be used as a source of resistance to southern blight in different geographical locations with diverse isolates of *S. rolfsii*. However, trials including more variable isolates are warranted to confirm this assumption.

Another possibility is that the resistance to southern blight could be controlled by multiple loci, with resistance to various isolates governed by different genes. This could

explain why 5913M was found to be resistance to only two isolates while PI 126932 was resistant to all three isolates.

Out of all the $F_1$ plants tested, 75% were able to survive indicating that the resistance could be dominant however looking at the frequency distribution it seems that some of the $F_1$ plants were more resistant than PI 126932 while some of them were behaving more like PI 126932. Such two peaks in frequency distribution could not be completely explained by a single gene model. Also, frequency distribution of $F_2$ was found to be skewed towards left indicating that resistance is recessive, which is opposite of that seen in $F_1$ population. This variation suggests that the disease is not conditioned by a single recessive or dominant gene and could in fact be governed by more complex genetics which could not be fully explained by this study, since only a few $F_3$ families were tested and a backcross of the $F_1$ to a donor parent was not tested in this study. Other possibilities also exist that could be responsible for such results, for example, compromised resistance in $F_1$ plants due to excess inoculum load (appendix D), less accurate disease rating scale, or unfit plants. In the $F_1$ plants, more individuals had a lower disease score as compared to the resistant parent PI 126932, which could potentially be a heterotic effect of the interspecific cross.

However, variation in the aggressiveness of *S. rolfsii* isolates along with variation in the inheritance pattern in crosses involving different resistant and susceptible sources could explain the deviation of the current results from the monofactorial model proposed by Mohr (1955). Earlier research showed that along with the variation in the pathogenicity among the isolates, variation also existed with respect to the mode of inheritance in different species. In *Capsicum annuum* L. the mode of resistance was

found to be inherited as a single recessive gene (Fery and Dukes, 2005). While it was found to be quantitative in alfalfa (Inami and Suzuki, 1981; Inami *et al.*, 1986; Pratt and Rowe, 2002). Such kind of variable mode of inheritance has also been reported for resistance to early blight of tomato caused by the fungus *Alternaria solani* Sorauer. The inheritance of resistance to early bright was reported to be quantitative and recessive in some lines (Barksdale and Stoner, 1977), but partially dominant, with epistasis in others (Nash and Gardner, 1988a). Such variation in the parental lines and fungal isolates could give rise to dissimilarity in the observed inheritance pattern.

The appearance of susceptible plants in PI 126932 and the $F_1$ could be due to incomplete penetrance. Incomplete penetrance can also perhaps explain the variation observed in the $F_3$ generation by Mohr (1955) and also in the inheritance study of resistance to southern blight in pepper conducted by Fery and Dukes, 2005. The incomplete penetrance effect in the resistance against tomato yellow leaf curl virus derived from *S. pimpinellifolium* was reported by other authors in various studies involving different accessions belonging to this species (Hassan, 1984; Pérez de Castro, 2007) indicating that the incomplete penetrance effect showed by the genes derived from accession of *S. pimpinellifolium* is not rather surprising. Also complexity in the genetics has also been reported for the resistances which have been derived from different lines of *S. pimpinellifolium* species (Kasrawi and Mansour, 1994; Hassan, and Abdel-Ati, 1999; Pérez de Castro, 2007). Incomplete penetrance has also been reported for *Fusarium oxysporum* f.sp. lycopersici in tomato (Retig *et al.*, 1967; Alon *et al.*, 1974) and for tomato spotted wilt virus in tomato (Salvador Roselló, 1998). It is quite possible that the resistant plants in Fla. 7776 could be just escapes rather than due to

incomplete penetrance effect of the genes involved. Also, susceptible plants in PI 126932 and the $F_1$ could be from excess inoculum load, due to variation in the colonization of the rye seeds by the fungus. Based on this study it would be hard to clearly justify whether such results are due to incomplete penetrance or merely due to escapes. However, incomplete penetrance could be confirmed by conducting a progeny test in $F_3$ families and also by inoculating near isogenic lines carrying genes conferring resistance.

An attempt to screen younger (4 week old) seedlings of both PI 126932 and Fla. 7776 resulted in death of all the plants, confirming the results of Mohr and Watkins (1959) that resistance in PI 126932 is not effective until plants are six weeks or older. This showed that resistance in PI 126932 is associated with physiological maturity of the plants. It was also found that the resistance was affected by environmental conditions (appendix D). Moreover, from this study it seems that the resistance could be controlled by more than one gene indicating that the resistance could have multifactorial inheritance. Such confounding factors together with the complex genetic nature of the resistance could be a reason to the limited success achieved in breeding for southern blight resistance using traditional breeding approaches. Thus, new strategies are needed for the identification and effective transfer of genes for southern blight in tomato.

The use of molecular markers to assist with plant selection is an alternative approach that could be adopted instead of conventional breeding while dealing with complex traits such as southern blight. The chromosomal position of gene(s) or quantitative trait loci controlling such complex traits can be determined by using molecular markers. The use of molecular markers can also facilitate an understanding

of the interaction between genes controlling the same trait. Once molecular markers linked to the gene(s) of interest are identified, they can be used to transfer the desired gene(s) into commercial varieties in less time as compared to conventional breeding (Sleper and Poehlman, 2006). The efficiency of developing superior tomato varieties with resistance to southern blight could be increased through marker assisted breeding.

Table 2-1. Determining inoculum load for differentiating southern blight resistant (PI 126932) and susceptible (Fla. 7776) tomato line. Plants were inoculated at an age of 8 weeks after sowing. Rye seeds colonized with GCT-1 isolate of *S. rolfsii* were used to inoculate the plants.

| Treatment | PI 126932 | | Fla. 7776 | |
|---|---|---|---|---|
| | Alive | Dead | Alive | Dead |
| Control[x] | 24 | 0 | 24 | 0 |
| 1g[y] | 23 | 1 | 8 | 16 |
| 1g[y] + met[z] | 24 | 0 | 8 | 16 |
| 2g[y] | 24 | 0 | 2 | 22 |
| 2g[y]+ met | 18 | 6 | 0 | 24 |

[x] Control treatment included 2 g non-inoculated rye seeds + 0.5% methanol spray.
[y] *S. rolfsii* (GCT-1) inoculated rye seeds.
[z] 0.5% Methanol spray.

Table 2-2. Two-way analysis of variance based on the ranked data of southern blight disease severity on tomato line PI 126932, Fla. 7776 and 5913M caused by GCT-1, WM609 and DF/LA-SR1 isolates. Data combined across two independent experiments.

| Effect | df[x] | df[y] | F-value | *P*- value |
|---|---|---|---|---|
| Tomato | 1.95 | 171 | 53.26 | < 0.001 |
| Isolate | 1.96 | 171 | 17.19 | < 0.001 |
| Tomato x Isolate | 3.81 | ∞ | 5.68 | < 0.001 |

[x] Numerator degrees of freedom.
[y] Denominator degrees of freedom.

Table 2-3. Relative marginal effects with 95% confidence interval estimated based on ranked data by two-way ANOVA type statistics for the severity of southern blight on three different tomato line caused by three different *S. rolfsii* isolates.

| Tomato line[x] | Isolate | Median[z] | Mean Ranking | RME[y] |
|---|---|---|---|---|
| Fla. 7776 | GCT-1 | 4.0 | 150 | 0.75 ± 0.072 |
| | WM609 | 4.0 | 138 | 0.69 ± 0.083 |
| | DF/LA-SR1 | 4.0 | 140 | 0.70 ± 0.094 |
| 5913M | GCT-1 | 3.5 | 127 | 0.64 ± 0.090 |
| | WM609 | 1.0 | 39 | 0.19 ± 0.086 |
| | DF/LA-SR1 | 2.0 | 84 | 0.41 ± 0.095 |
| PI 126932 | GCT-1 | 2.0 | 80 | 0.40 ± 0.060 |
| | WM609 | 1.5 | 53 | 0.26 ± 0.062 |
| | DF/LA-SR1 | 2.0 | 84 | 0.42 ± 0.084 |

[x] Data pooled for two independent experiments.
[y] Relative Marginal Effect with 95% confidence interval.
[z] Score higher than 2.5 indicates susceptibility to specific *S. rolfsii* isolate.

Table 2-4. Statistical analysis of variance based on disease severity scores in PI 126932, Fla. 7776 and 5913M caused by GCT-1 isolate. Data combined across two experiments.

| Effect | df | F-Value | *P*- Value |
|---|---|---|---|
| Tomato lines | 2 | 20.19 | < 0.01 |
| Error | 63 | | |

Table 2-5. Relative marginal effects with 95% confidence interval estimated based on ranked data by ANOVA type statistics for the severity of southern blight on three different tomato line caused by GCT-1 isolate of *S. rolfsii*.

| Tomato line[x] | Isolate | Median[z] | Mean Ranking | RME[y] |
|---|---|---|---|---|
| Fla. 7776 | GCT-1 | 4.0 | 46 | 0.68 ± 0.072 |
| 5913M | | 3.5 | 37 | 0.55 ± 0.083 |
| PI 126932 | | 2.0 | 18 | 0.26 ± 0.060 |

[x] Data pooled for two independent experiments.
[y] Relative Marginal Effect with 95% confidence interval.
[z] Score higher than 2.5 indicates susceptibility to specific *S*. *rolfsii* isolate.

Table 2-6. Segregation for resistance to southern blight in parental, $F_1$ and $F_2$ populations.

| | Observed | | Expected | | Expected | | |
|---|---|---|---|---|---|---|---|
| Population | R[v] | S[w] | R | S | ratio | $\chi^2$ | *P*-value |
| PI 126932 | 46 | 4 | 50 | 0 | | | |
| Fla. 7776 | 6 | 44 | 0 | 50 | | | |
| $F_1$ | 38 | 12 | 50 | 0 | | | |
| $F_2$ | 22 | 74 | 24 | 72 | 1:3[x] | 0.22 | 0.63 |
| | | | 60 | 36 | 1.6:1[y] | 64 | <0.01 |
| | | | 36 | 60 | 1:1.6[z] | 8.7 | <0.01 |

[v] Number of resistant plant ( wilting index ≤ 2.5).
[w] Number of susceptible plant ( wilting index > 2.5).
[x] Ratio for single recessive gene.
[y] Single dominant gene ratio adjusted for incomplete penetrance.
[z] Single recessive gene ratio adjusted for incomplete penetrance.


Figure 2-1. Southern blight symptoms on plants of parental lines. A) wilting symptoms on susceptible parent Fla. 7776, B) wilting symptoms on resistant parent PI126932, C) stem lesion on Fla. 7776, D) stem lesion on PI 126932.

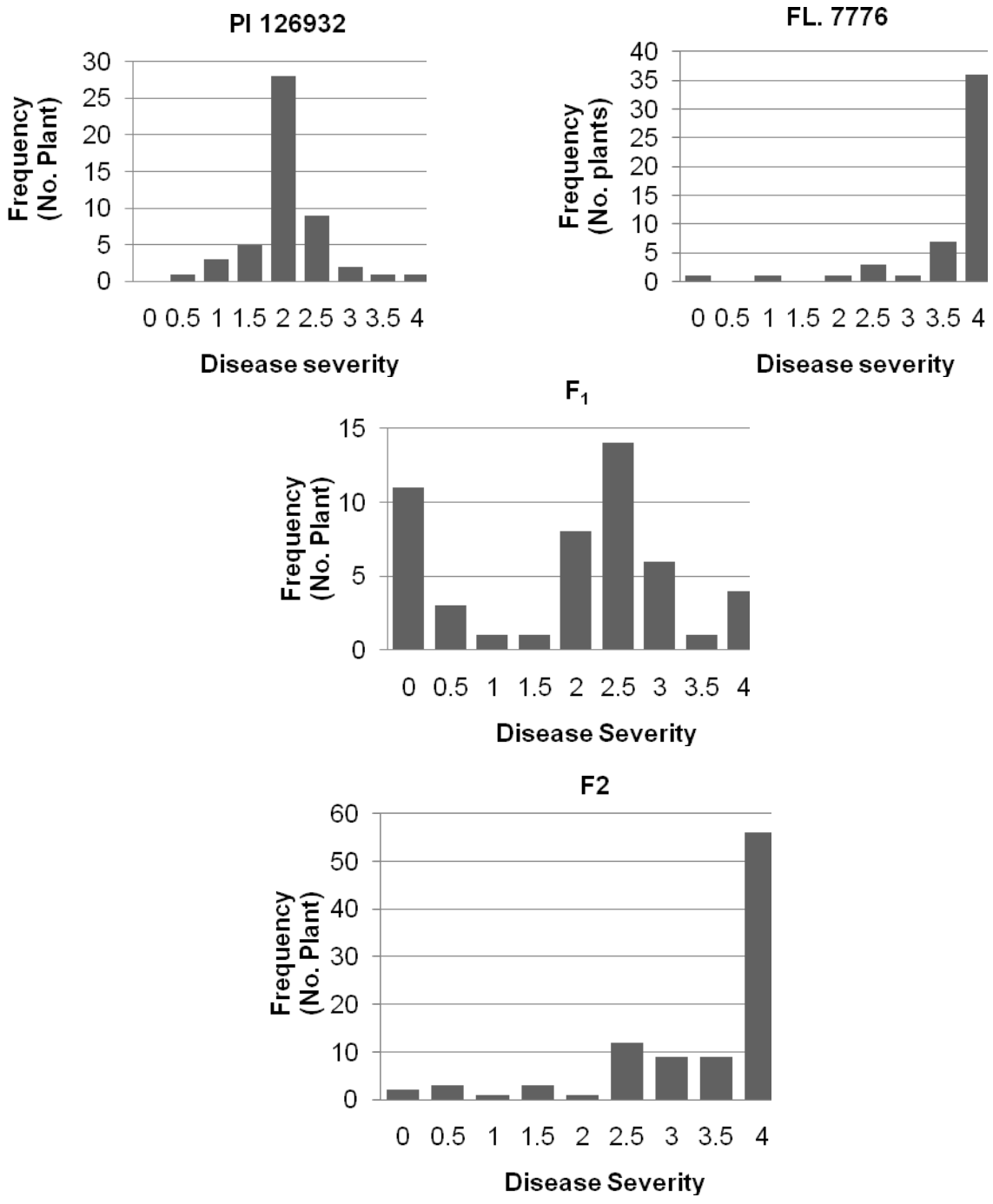


Figure 2-2. Frequency distribution of southern blight disease severity for plants of tomato line PI126392, Fla.7776, and $F_1$. 0 = no wilting symptoms with initiation of stem lesion, 0.5 = initiation of wilting, 1 = 12.5% of total leaf area showing wilting symptoms, 1.5= 12.5 - 25% wilting, 2= 25% - 37.5% wilting, 2.5 = 37.5%- 50% wilting, 3 = 50%-75% wilting, 3.5 = >75% wilting with lodged plant, 4 = Dead plant. Rating >2.5 suggest susceptible plants.

# CHAPTER 3
# IDENTIFICATION OF MOLECULAR MARKERS LINKED TO SOUTHERN BLIGHT RESISTANCE IN TOMATO

## Introduction

Southern blight of tomato (*Solanum lycopersicum)* is caused by *Sclerotium rolfsii* Sacc. (Sherf and MacNab, 1986). Economic losses caused by this disease to the tomato industry in Florida could increase in the near future due to the loss of Methyl Bromide as a soil fumigant and a lack of satisfactory alternative control methods. Host resistance is an economical, environmentally safe, and efficient means to control disease. Mohr (1955) searched for southern blight resistance in a screen of several varieties of *Solanum lycopersicum* as well as a number of lines from the wild relative *Solanum pimpinellifolium* and *Solanum peruvianum.* Out of the 30 lines tested, Mohr categorized a single line from *Solanum pimpinellifolium* accession as resistant against southern blight. The line came from Pampa de Matacabilles, Peru, and was introduced by the Plant Introduction Service of the U.S. Department of Agriculture as PI 126932.

In order to verify that PI 126932 is in fact a suitable genetic source for resistance against southern blight in tomato, studies were carried out at the University of Florida, IFAS, Gulf Coast Research and Education Center, Wimauma, FL. In this study the PI 126932 was inoculated with three different strains of *S. rolfsii* and results were compared with a susceptible inbred line Fla. 7776. It was found that PI 126932 was resistant against all three *S. rolfsii* strains tested. Based on the results it was confirmed that PI 126932 was a good source for deriving genetic resistance against southern blight in tomato, similar to previous studies (Mohr, 1955).

Along with PI 126932, other reported sources for southern blight resistance were six breeding lines (5635M, 5707M, 5719M, 5737M, 5876M and 5913M) released from

Texas A&M University, containing *S. pimpinellifolium* in their pedigree (Leeper *et al.*, 1992). Breeding line 5913M was tested along with PI 126932 and Fla. 7776 against the three isolates. 5913M was found to be susceptible to a field isolate obtained from Florida (GCREC), but resistant against two isolates obtained from Georgia and Louisiana. This suggested that the resistant breeding lines from Texas A&M may not be a reliable source of resistance to *S. rolfsii* isolates from Florida.

Because the resistance source, PI 126932 is a wild species, the most effective way of transferring resistance to cultivated tomato lines is through backcrossing. One advantage of *S. pimpinellifolium* being the source is that the transfer of resistance from *S. pimpinellifolium* to *S. lycopersicum* is rather straightforward through conventional breeding since both species are closely related and hybridize easily (Rick, 1958). Backcrossing has long been a valuable strategy in plant breeding for a number of crops. Backcrossing is a type of recurrent hybridization in which the main aim is to substitute a desirable allele(s) for the trait of interest in a desirable cultivar without losing or changing the existing genetic background of the that cultivar except for the substitution of desired allele(s).

Semagn *et al*.(2004) pointed out that normally during backcross breeding it is expected that the genome of recurrent parent will be recovered at a rate of $1-(1/2)^{t+1}$, for "t" generations of backcrossing. Thus with the completion of the fourth backcrossed generation we expect to have recovered 96.87% of the recurrent parent's genome. However, in actual practice, this theoretical percentage value is hardly achieved especially when dealing with polygenic trait. One reason for recovering less than the theoretical percentage is linkage drag, which refers to the reduction of fitness in a

recurrent parent due to introduction of deleterious genes along with the beneficial gene during backcrossing (Semagn *et al.*, 2006). Young *et al.* (1988) suggested that based on linkage distance the unwanted DNA segment can be removed through additional backcrossing, but to achieve significant progress, many generations are required.

Molecular markers offer a tool by which the amount of donor genome transferred can be controlled during each backcross generation. Use of marker assisted breeding techniques can speed up recovery of the recurrent parent's genome and thereby improve efficiency of transferring gene(s) from the donor parent. Markers can aid in minimizing the linkage drag associated with the target gene, and reduce the number of generations required to recover a high percentage of the recurrent parent genotype. Chahal and Gosal (2002) suggested that applying a molecular marker assisted techniques to a backcross breeding program could reduce linkage drag by at least tenfold in a fraction of the time required in traditional backcross breeding. This technology can also help to gain better knowledge about the genetics of southern blight resistance in tomato, which will help in transferring resistance into desired line.

Earlier study showed that the plants were not fully resistant till the age of six to eight weeks and was greatly influenced by environmental conditions (Mohr, 1955). Along with this, our study regarding the inheritance of southern blight resistance in tomato showed that resistance was probably conferred by more than one gene indicating multifactorial inheritance. Such influencing factors together with the complex genetic of the resistance could greatly affect the selection in a traditional breeding approach. Molecular markers and marker assisted selection technology is an alternative

system which could help to validate and effectively transfer genes conferring southern blight resistance to tomato.

However in order to utilize the benefit obtained from marker assisted selection one requires quality markers that are closely linked to the trait of interest. PI 126932 is a convenient resistant source for identifying associated markers since many polymorphic markers have already been reported by Hutton (2008) between PI 126932 and Fla. 7776.

The primary objective of this study was to identify PCR-based molecular markers linked to loci conferring resistance to southern blight.

## Materials and Methods

### Plant Materials

Seeds for the resistant accession PI 126932 and a susceptible inbred line Fla. 7776 were grown and crosses were made between them in fall 2008 under greenhouse conditions with Fla. 7776 serving as a recipient parent. Subsequently an $F_2$ population was generated by self pollination of $F_1$ generation in spring 2009. In the spring of 2009 backcrossing was carried out to obtain the $BC_1$ generation.

In order to study the response of PI 126932, Florida 7776, $F_1$, $F_2$ and $BC_1$ to southern blight, 50 seeds of PI 126932, Florida 7776, $F_1$, and $BC_1$ as well as 96 seeds of $F_2$ were sown in 128-wells styrofoam Speedling® trays (3.8 $cm^3$ cell size) containing peat-lite mix (Speedling Inc., Sun City, FL) in fall 2009. The plants were fertilized with compound fertilizer 20-20-20 (N-$P_2O_5$-$K_2O$) at an interval of 5 days. After four weeks, seedlings were transplanted into four inch diameter pots and maintained in the greenhouse for the duration of the experiment. A complete randomized block design was selected for this study.

For the marker study in the $F_2$ population, three groups containing two batches of 96 $F_2$ seeds and 12 seeds of both the parents were sown in the speedling trays with an interval of three weeks in June 2009. All three groups were transplanted and grown as mentioned earlier. All the groups were inoculated at an age of 8 weeks. Progeny of $F_2$ selections were evaluated in subsequent experiments.

**Genomic DNA Extraction from Leaves**

Genomic DNA was extracted buy using a protocol described by Fulton (1995) with following modifications. About 3-4 new leaflets of about 1.5 cm size were collected in 1.2 mL library tubes (VWR, West Chester, Pennsylvania) and frozen at -80 $^{o}$C. The frozen leaflets were homogenized using a Talboys high throughput homogenizer (Henry Troemner LLC, Thorofare, New Jersey). DNA was extracted from the frozen leaf tissue by incubating each sample with 166 µL of DNA extraction buffer (0.35 M sorbitol, 0.1 M tris-base, 5 mM EDTA, pH 7.5,1.3mg sodium bisulfate ), 166 µL of nuclei lysis buffer (0.2 M tris, 0.05 M EDTA, 2 M NaCl, 2% CTAB), and 66 µL of sarkosyl (5%w/v) at 65 $^{o}$C followed by a chloroform : isoamyl alcohol (24:1,v:v) extraction and a final nucleic acid precipitation with ice cold isopropanol. DNA was pelleted by centrifugation at 4000 rpm for 15 min and rinsed with 70% ethanol. After air drying samples overnight, the DNA was re-suspended in 100 µL nuclease free water and stored at -20 $^{o}$C. Each sample yielded 300-600 ng/µL of DNA. The DNA was diluted down to 20 ng/µL for molecular marker study.

**Inoculation and Disease Evaluation**

The *Sclerotium rolfsii* isolate GCT-1 used in this study was obtained from naturally infected tomato fields at GCREC, Balm. The fungus was maintained on PDA plate at 25 $^{o}$C. For inoculum preparation 200 g of rye seeds were sterilized in 1000 ml Erlenmeyer

flask with 300 ml water and inoculated with approximately ten 9 mm PDA plugs containing both mycelium and mature brown sclerotia. The rye seed cultures were incubated at room temperature (25-30 $^{o}$C) for a period of 3-4 weeks.

The 8 weeks old plants were inoculated by spreading 2 g of colonized rye seeds on the soil surface close to the base of the tomato plant. After 48 hr the inoculated pots were examined to visually confirm the emergence of mycelial growth from the colonized rye seeds. The pots not showing any sign of mycelial growth were re-inoculated. Disease progress was recorded for individual plants every alternate day after inoculation, using a visual scale of 0-4 with increasing severity: 0 = no wilting symptoms with initiation of stem lesion , 0.5 = initiation of wilting, 1 = 12.5% wilting of total leaf area, 1.5 = 12.5 - 25% wilted leaf area, 2 = 25% - 37.5% wilting, 2.5 = 37.5%- 50% wilting, 3 = 50%-75% wilting, 3.5 = >75% wilting and stem collapse, 4 = Dead plant. Plant with no clear sign of stem lesions was considered as an escape and was excluded from the study.

**Molecular Markers and $F_2$ Genotyping**

To detect polymorphisms between PI 126932 and FL. 7776 about 252 PCR based DNA markers spanning the tomato genome were screened. Out of this, 115 markers had been earlier reported to be polymorphic among PI 126932 and FL.7776 (Hutton, 2009). Markers were selected such that any two adjacent markers on a given chromosome were not more than 20 cM apart. Most of the markers were obtained from the Solanaceae Genomics Network (SGN) (http://www.sgn.cornell.edu) which included CAPS, SCAR and SSR markers, or were obtained from various published sources (Suliman, 2002; Yang, 2004; Dynze, 2007; Ji *et al.*, 2007; Hutton, 2008).

Genomic DNA from parents, $F_1$, $F_2$, $BC_1$ and $F_3$ population were extracted prior to inoculation. Plants were rated on 21$^{st}$ day after inoculation. Plants rated with a score of 2.5 or below where considered resistant plants as these plants were able to recover from the infection. While the plants with score greater than 2.5 were considered to be susceptible plants since they were never able to recover and eventually died. Selective genotyping system was used to genotype $F_2$ individuals found on the extreme ends of a frequency distribution based on disease severity. About 23 extreme resistant and 22 susceptible $F_2$ individuals were selected for selective genotyping. As a control, an individual DNA sample from both the parents and the $F_1$ were also included in the screening study.

Amplification of CAPS and SCAR markers was performed in 10 µL reactions containing 50-60 ng of DNA, 2 µL of 20% Dimethyl sulfoxide (DMSO), 1 µL of 25 mM of dNTPs, 0.8 µL of 25 mM $MgCl_2$, 1 µL of 10x PCR buffer, 0.8 µL of forward and reverse primer (5 µM) and 0.08 µL of Taq polymerase (5 u/µL) (New England Biolab, Ipswich, Massachusetts). Annealing temperatures for each marker was optimized using a Mastercycler ep gradient (Eppendorf AG, Hamburg, Germany). The annealing temperature was selected to yield a sufficient amount of PCR product at the desired range. Separation of bands was either done on an agarose gel (2%, 3% or 4%) or on 4% MetaPhor® agarose (Lonza Rockland, Inc., Rockland, Maine) depending upon band size and percentage by which the DNA fragments differed. PCR products were run on agarose or metaphor agarose gels in 1x TBE at 120 V for 90 min for visualization. Most of the SSR and InDel markers were scored on denaturing polyacrylamide gel using LI-COR 4300 DNA Analyzer system (LI-COR Biosciences, Lincoln, Nebraska).

Amplification of SSR and InDel markers was performed in 10 µL reactions containing 20 ng of DNA, 0.8 µL of 2.5 mM of dNTPs, 0.6 µL of 25 mM $MgCl_2$, 0.04 µL of forward primer (5 µM) with 5´ M-13 tail, 0.4 µL reverse primer (5 µM), 0.18 µL fluorescent M-13 tail (10µM), 1 µL of 10x PCR buffer and 0.05 µL of Taq polymerase (5u/µL). For detection on polyacrylamide gel, PCR was carried out in Mastercycler ep gradient with the following program: Step 1) 2 min 95 $^{o}$C, Step 2) 7 cycle of 45 sec 95 $^{o}$C to 68 $^{o}$C (with 2 $^{o}$C drop per cycle), and 1 min 72 $^{o}$C, Step 3) 28 cycle of 1 min 72 $^{o}$C, 45 sec 95 $^{o}$C, 45 sec 50 $^{o}$C and 1 min at 72 $^{o}$C, and Step 4) final extension of 5 min at 72 $^{o}$C. Amplified products were run on 6.5% polyacrylamide gel in 1x TBE buffer at 1400v for 120 min for visualization.

**Marker Analysis**

The chi-square ($\chi^2$) method was used to test the goodness of fit for observed ratios to theoretically expected ratios of marker scores in all populations. For the marker analysis approach, selections were grouped as resistant or susceptible, and marker data were scored on the basis of the probability of a resistant allele (2, 1, and 0) for co-dominant markers. Detection of linked markers was carried out by using WinQTLCart (Wang *et al.*, 2010) through t-tests based on single marker analysis (Soller *et al.*, 1976). QTL analysis was performed with single marker analysis rather than using interval mapping since the number of available markers was limited and large distances between some markers did not allow for a reliable placement of potential QTLs. Markers showing significant linkage were confirmed by genotyping additional $F_2$ individuals. Selected $F_2$ progenies were also genotyped with significantly linked markers.

## Results

A screen of 252 PCR based DNA markers including 115 markers that were previously reported to be polymorphic between PI 126932 and Fla. 7776 (Hutton, 2009 were tested for polymorphism between two parents, including an $F_1$ to test for co-dominance (Table C-1). Out of the 252 markers 152 were polymorphic and 127 were co-dominant. Tightly clustered markers were eliminated leaving 102 markers that were used to genotype the $F_2$ population (Table 3-1). The 102 markers spanned the tomato genome with a maximum distance between markers of 30 cM, a mean distance of 12 cM, and a median distance of 10 cM.

From the extremes of the phenotype distribution, 23 resistant $F_2$ individuals and 22 susceptible individuals were selected for genotyping. Based on single marker regression analysis three loci were detected, one on chromosome 4, one on chromosome 10, and another on chromosome 11 (Table 3-1). Markers at the three detected loci were tested on an additional 45 resistant and 45 susceptible individuals and the significant association was maintained for the markers at loci on chromosomes 10 and 11, but marker at the chromosome 4 loci was not significantly associated with resistance (Table 3-2).

The significance of the association at the two remaining significant loci was confirmed by genotyping and phenotyping of an additional 354 $F_2$ plants (Table 3-2). The markers SL10105i on chromosome 10 and T0408-1, 2 on chromosome 11 segregated as per the expected 1:2:1 ratio indicating no segregation distortion (Table C-2). While the PI 126932 allele at the chromosome 10 locus (L1) was associated with increased resistance, the PI 126932 allele at the locus on chromosome 11 (L2) was associated with increased susceptibility to *S. rolfsii* (Table 3-3).

Evidence of both overdominant and epistatic effects at both loci was observed among $F_2$ segregants (Table 3-3). The apparent gene action at each locus was dependent on the allele state at the other locus. When L1 was homozygous for the PI 126932 allele, the Fla. 7776 allele at L2 appears to be dominant for resistance, however when L1 was heterozygous, a high level of resistance is only observed when L2 was heterozygous (behaving overdominant). When L1 was homozygous for the Fla. 7776 allele, no allele state at L2 provides a high level of resistance. When the L2 allele was homozygous for PI 126932 then all allele states at L1 were susceptible. When L2 was heterozygous for the PI 126932 allele at L1 appears to have a dominant or slightly overdominant resistance effect. Finally, when L2 was homozygous for the Fla. 7776 allele, the Fla. 7776 allele at L1 appeared to have a recessive resistance effect.

This unusual pattern of overdominant and epistatic effects was also observed in a separate experiment using $F_3$ family individuals (Table 3-4). When L1 was homozygous for PI 126932 allele the highest number of resistant plants were observed when L2 was homozygous for Fla. 7776 allele. As seen in $F_{2,}$ high level of resistance was observed in $F_3$ plants when both L1 and L2 were heterozygous (overdominant effect). When L1 was homozygous for Fla. 7776 allele, no allelic combination on L2 showed good resistance level. The overdominant and epistatic effects in the $BC_1$ population were consistent with those seen in the $F_2$ and $F_3$ populations (Table 3-5). High level of resistance was observed in $BC_1$ when both loci were heterozygous. The marker-trait associations were confirmed in a $BC_1$ population using single marker analysis (Table 3-6).

## Discussion

This study identified molecular markers linked to two loci one chromosome 10 and another on chromosome 11 controlling resistance to southern blight in tomato. The

pattern of phenotypic data observed in the $F_2$ generation exhibited a non-continuous distribution, indicating that resistance was inherited as a qualitative trait controlled by 1 to 2 genes rather than a quantitative trait controlled by multiple genes. It is possible that there are additional genes influencing resistance, but were not detected in this study because of gaps in genomic coverage of molecular markers or due to the lack of statistical power to detect loci with weaker effects.

Based on the results of marker analysis for $F_2$ and $F_3$ plants, many of the resistant plants were heterozygous at both the loci. Heterosis due to true- or pseudo-overdominance could possibly explain why some of the $F_1$ and $F_2$ plants exhibited greater resistance than the resistant parent itself. The over-dominance hypothesis postulates the existence of loci at which the heterozygous state is superior to either homozygote. Pseudo-overdominance, in contrast, refers to the situation of linked genes with favorable dominant alleles linked in repulsion. However, clear differentiation could not be made between true- or pseudo overdominance from the $F_2$ and $F_3$ generation. Parvez (2006) pointed out that linkage was a major factor in preventing one from differentiating overdominance from pseudo-overdominance. Budak *et al.* (2002) referred that pseudo-overdominance is a condition that gives the false presumption of overdominance, in which a pair of linked loci would behave as a single locus showing overdominance effect eventually skewing the measure of true overdominance.

Regardless of the mechanisms involved, the genes at loci L1 and L2 identified in this study provide a good source of resistance that could be introgressed into commercial cultivars. Even though these loci do not confer complete resistance, it should be considered that the disease pressure in the field is generally less than the

conditions in our disease assay. Thus the partial resistance conferred by the identified genes may in practice be sufficient to efficiently protect tomato against *S. rolfsii*. Although the locus L1 found on chromosome 10 did not confer complete resistance, its dominant effect, as observed in the $F_2$ dataset, makes it an interesting candidate for breeding resistance into $F_1$ hybrids, since introgression in only one parent is necessary.

Our study showed that southern blight resistance in tomato as conferred by genes L1 and L2 is influenced by the overdominance effect and epistasis between the detected loci. Both of these phenomena affect the development of resistant cultivars through conventional breeding. Sofi *et al.* (2007) indicated that epistasis affects the estimates of expected gain under selection during conventional breeding and also reduces the efficiency in estimating additive and dominance component of genetic variance. Recent improvement in methods for QTL-mapping with use of molecular markers has provided an opportunity to detect and understand the effect of epistasis between QTLs controlling complex trait (Carlborg and Haley, 2004). Such methodologies can be used in better understanding the genetics of southern blight in tomato which will eventually be helpful in generating resistant cultivars. Also, selection of heterozygous plants to take advantage of the overdominance effect would be possible using linked markers. However if the heterotic effect is due to pseudo overdominance then the heterozygous effect is fixable and superior inbred to hybrid can be obtained. This could also be facilitated by tightly linked markers which would enable the detection of a recombination event converting repulsion into coupling phase linkage that upon selfing could yield a fixable heterotic effect.

The conventional approach for breeding southern blight resistance in tomato by phenotypic selection could be negatively affected by the complexity of this trait. Since some of the resistant plants were found to be heterozygous at both the loci, selection under conventional breeding could result into plants segregating for southern blight resistance. However, some of these issues can be resolved by utilizing alternative approach i.e. molecular breeding. Associated molecular markers can not only help in the selection process but also facilitate a better genetic understanding of this disease in tomato which would help in developing southern blight resistant tomato lines and could improve the resistance level in other host species.

Table 3-1. Polymorphic markers for Fla. 7776 and PI 126932. Significant *P*- value indicates association with QTL.

| Marker | Approx. Position [x] | Forward primer | Reverse primer | *P*- value[y] |
|---|---|---|---|---|
| CosOH47 | 1.010 | ttgctgattttcttcccatttt | gcagctggagtgagaggaac | 0.164 |
| LEOH36 | 1.017 | tcacaaaaatggcgatgaga | ccacctgtggatccttgact | 0.278 |
| C2_At5g18580 | 1.035 | tgccacattgcctctgtatgtacagaac | atgtcaattcgggcttgagtaagtg | 0.880 |
| SSR95 | 1.043 | caatccaacaagcaatccct | ccacataactaagcccacaactt | 0.306 |
| SSR 316 | 1.053 | ccaccgcaacaaaccttatt | gggtggtgagaaggatctga | 0.628 |
| SL20268i | 1.061 | cactccgttccttggcatac | cccttccgttcttaaatacttg | 0.928 |
| SL10975i | 1.070 | gtgaacccggaactctgaac | tcattgccacacagaagcag | 0.894 |
| LEVCOH11 | 1.085 | caaccatgttagatgtgccagt | taagagaggggaatggtgatgt | 0.981 |
| C2_At3g04710 | 1.095 | agggtgcagatcctgcaatacccag | tccagcctcactttgtaaatcaacatc | 0.416 |
| SSR42 | 1.107 | ccatggcttcgttatcccta | taaaggtgaaggaacgggtg | 0.599 |
| SL10126i | 1.137 | atgactgagcatctgcgttc | gccgccacttattgtaggat | 0.492 |
| TOM11 | 2.013 | ttgtaatggtgatgctcttcc | cagttactaccaaaaatagtcaaacac | 0.224 |
| SL10682i | 2.034 | gccgctcgtacaaggttattc | tcgatttcccaaattgaagc | 0.457 |
| SSR96 | 2.043 | gggttatcaatgatgcaatgg | cctttatgtcagccggtgtt | 0.943 |
| SSR5 | 2.050 | tggccggcttctagaaataa | tgaaatcacccgtgaccttt | 0.863 |
| LEOH348 | 2.064 | tgtttcccttcattcatgct | ccaattggataaattggtggt | 0.421 |
| LEOH113 | 2.075 | aaacagaggtgccgaagaaa | gagctacaagcagcaaacca | 0.602 |
| C2_At5g66090 | 2.083 | atctctctgagggttcaagacagg | tatatcagctccatacttctttgc | 0.862 |
| LEOH174 | 2.096 | cgagtccgaggaagactgat | tcaagacagacacggattgc | 0.749 |
| TG525 | 3.014 | tatcagttcacctcccagca | gccaatcatgtgaatggtgat | 0.179 |
| LEOH124i | 3.021 | ccgtctccttctccctcttt | ctggctggtgtcttctccat | 0.348 |
| SL10480i | 3.038 | tcgcatcaattgcaacacac | aaacgcaaggtgatcagtcc | 0.617 |
| LEOH223 | 3.040 | acaagagtcgggtgatggac | gcgatggaaatagcatcaca | 0.291 |
| C2_At1g02140 | 3.054 | tccgttatgctaacaattccaac | tgtgttcatttcccatcacaatctc | 0.313 |
| FEY | 3.064 | accgcttcctctatcaagca | atgccgaataaccaagcaac | 0.833 |

[x] approximate position obtained from Dynze, *et al.*, 2007.

Table 3-1. Continued

| Marker | Approx. Position [x] | Forward primer | Reverse primer | *P*- value |
|---|---|---|---|---|
| C2_At5g60160 | 3.068 | acacaatgctaatcaacgttatgc | tcatccaccgcgcacatttc | 0.859 |
| C2_At5g52820 | 3.070 | tgggatctaaatacccagacacc | acagaaagaacccaatttctgtgc | 0.781 |
| C2_At1g61620 | 3.087 | atgcattctagaatgccttttgtc | tccctggctttctgcagcatc | 0.396 |
| LEOH127 | 3.098 | caaggcatcaacctaattgga | tgtaggcttgaaaaataagaggaga | 0.197 |
| SSR296 | 4.010 | ccggaacaagtcccttcata | tcagccaagttcatggtacatc | 0.040* |
| SL10255i | 4.025 | tttgctgtatgtatatgctcttcc | gcactctgataaaagacttgcag | 0.929 |
| C2_At3g17040 | 4.032 | tggggttggatggagtggaaag | agtagaggttacgaatttcctctgc | 0.718 |
| SSR603 | 4.045 | gaagggacaattcacagagtttg | ccttcaacttcaccaccacc | 0.515 |
| SSR306 | 4.053 | acatgagcccaatgaacctc | aaccattccgcacgtacata | 0.222 |
| C2_At1g71810 | 4.072 | tcatgcagatccacatcctggaaac | agtgacaaaatccttggccaatgc | 0.465 |
| CT194 | 4.079 | tgggttttctgggtatggaa | gcatgatgggcagtctgtaa | 0.233 |
| SL00030 | 4.087 | agttggtggtggtagtgaag | ttctctccattcacagttcc | 0.308 |
| SSR146 | 4.107 | tatggccatggctgaacc | cgaacgccaccactatacct | 0.190 |
| TG441 | 5.008 | tggtatggtaccacgacaaag | ttttcaggtcgaggataccg | 0.443 |
| P11M6 | 5.015 | gaggtaggacttagaaaacata | aatcaacaccactaaatgcaga | 0.303 |
| Bs4 | 5.022 | ggagctgaatacggattgga | atcgttccgatgatttctgg | 0.537 |
| CT93 | 5.030 | ttctgaggttggctgagaccttgt | tctggtagacaatggaaccgcctt | 0.506 |
| SL20210i | 5.046 | gaggtatcaaagttatgctttcac | tcgattagttgagctagttattcc | 0.625 |
| C2_At1g14000 | 5.060 | agcgttacatggctggatcgatg | atacgtctttaacaattcaatcatgc | 0.503 |
| TOM49 | 5.068 | aagaaacttttgaatgttgc | attacaatttagagagtcaagg | 0.878 |
| CosOH73 | 5.082 | cttcccgacaagcacaaaaa | cgaatgctctgtaccatttcc | 0.979 |
| SSR162 | 5.090 | gctctctacaagtggaactttctc | caacagccaggaacaaggat | 0.721 |
| SL10328i | 6.000 | accgtgaatctgaggttgct | cgtgccaatgtccaactaag | 0.097 |
| T1456 | 6.010 | tagcttctgccattgatttgagc | tgagagggaagtatctgtatgccc | 0.252 |
| p55P11 | 6.020 | tcccaaaccccaacttaaaa | ggacggtctgtgagtggaat | 0.252 |

[x] approximate position obtained from Dynze, *et al.*, 2007.

Table 3-1. Continued

| Marker | Approx. Position [x] | Forward primer | Reverse primer | *P*- value |
|---|---|---|---|---|
| P6-25F2R5 | 6.025 | ggtagtggaaatgatgctgctc | gctctgcctattgtcccatatataacc | 0.578 |
| TG590f2R2 | 6.029 | acagcaggaggtgatggaatac | cgggtcgagcgatttgttta | 0.711 |
| T0834-Fla,R2 | 6.032 | ctgttaattgggaccccatcagaagcagg | ggaaggtgatgctgcaatccttcagataacc | 0.354 |
| C2_At1g44760 | 6.044 | ttcttcatctgctgctcatcttgc | agagggttttttctgacccaagac | 0.773 |
| SP | 6.068 | agggttgaagttcatggtgg | gatgttccctgagatatgga | 0.743 |
| LEOH112 | 6.078 | gccaattgaactgaccatctg | cccatgtatttggctgtagaa | 0.197 |
| SSR350 | 6.100 | ggaataacctctaactgcggg | cgatgccttcatttggactt | 0.224 |
| C2_At5g20180 | 7.006 | tgctatgtacatctaatcccaagcac | agctatccccctttccaccaag | 0.754 |
| C2_At1g19140 | 7.024 | aggcccttgtactcagtgcctctc | tcatggcggtttcagtccatcc | 0.320 |
| SSR276 | 7.033 | ctccggcaagagtgaacatt | cgacggagtacttcgcatt | 0.736 |
| TG217 | 7.043 | cgttgcttcctgatcctacc | agctagtgatgatcctggcg | 0.725 |
| TG174 | 7.052 | ttccaagatcttttagcgtctc | ctgttgcggatgtgatcatt | 0.614 |
| TG216-1 | 7.062 | gctttcggtactgcatcctc | taaatgaagcctgggattgc | 0.600 |
| C2_At1g56130 | 7.085 | acatatagctgttgggaacaggg | taggtttaaacttgcgaacatcc | 0.159 |
| C2_At5g46630 | 8.002 | tggcgcctttgatgaagatgc | agattttgagggtaaccaaagtcc | 0.390 |
| LEOH147 | 8.020 | agttcccgttggtgttcaag | cccttgccagtggatgttag | 0.820 |
| C2_At2g26830 | 8.030 | tcaaatctagatggttctcacttctctg | aagtgcgtgcatcaataaatgactg | 0.532 |
| TG302 | 8.038 | ctctccgggtggctattaca | tcttgggactcctccttttct | 0.239 |
| SSR38 | 8.053 | gtttctatagctgaaactcaacctg | gggttcatcaaatctaccatca | 0.412 |
| TG294 | 8.083 | gttctcattggagccatcgt | gattgggcacactcacttt | 0.838 |
| C09HBa0203J14.1 | 9.023 | gcatgactgctctcagttggcttt | ggcagcttcatttgagtgtggaga | 0.242 |
| SSR70 | 9.031 | tttagggtgtctgtgggtcc | ggagtgcgcagaggatagag | 0.531 |
| LEOH144 | 9.058 | atggcctaggattgcatctg | ttgcatacacttggataaaagca | 0.955 |
| LEOH170 | 9.074 | ggattagaagagaaaaacaaaagca | agccttctcaaattcctcctc | 0.981 |
| SSR333 | 9.100 | gttcccgcttgagaaacaac | ccaatgctgggacagaagat | 0.718 |

[x] approximate position obtained from Dynze, *et al.*, 2007.

Table 3-1. Continued

| Marker | Approx. Position [x] | Forward primer | Reverse primer | *P*- value |
|---|---|---|---|---|
| C2_At3g21610 | 10.002 | atgggattcaaaaaggatgcttagc | agcctaacaccagtagcatcatacattac | 0.120 |
| C2_At5g60990 | 10.014 | tgatacactgaagcagcagtatcg | agccagaagacgagttgcatcac | 0.029* |
| SL10105i | 10.030 | ccaagcccttctgatttagtg | ctttacataattggccgacaaac | 0.024* |
| LEVCOH15 | 10.037 | gcaaccaccaatgttcattaca | aagctaaatctggcttgtggag | 0.027* |
| SL10419i | 10.043 | ccttgattggaaaaagcaagac | gccattcttttgggagataaac | 0.040* |
| C2_At3g58470 | 10.061 | attgcttgtcccacactttatgc | tactgttcaaaccgtttgtcatactc | 0.533 |
| T1682 | 10.066 | cctccctcaccatccaataa | ctgctttaaccaccggattc | 0.531 |
| TG403(dCAPS)R | 10.082 | tttgccttggttcccttatgcagc | tacgtatttttgaaatatcctgttctttcag | 0.282 |
| SL10683i | 11.000 | tggatattcgtatattcgagacagg | attccgatccatccaatctg | 0.301 |
| TG497 | 11.004 | cgtctcgagaggaagtgagg | tactggcacccatgctacaa | 0.361 |
| SSR80 | 11.017 | ggcaaatgtcaaaggattgg | agggtcatgttcttgattgtca | 0.748 |
| T0408-1,2 | 11.026 | tagacggtgctcatgtcgag | gttctcggcacccattctaa | 0.04* |
| SSR76 | 11.046 | acgggtcgtctttgaaacaa | ccaccggattcttcttcgta | 0.815 |
| C2_At4g10050 | 11.054 | tcctgttgaaagttcaatctgtgt | ctccactcatgtcacaaacca | 0.416 |
| SL10737i | 11.060 | ccactcctgggactcaaatc | tggacccacaggtaatgagg | 0.256 |
| cLET-24-J2 | 11.073 | caaccatcctagcaatgaaatct | gaggcattcactctcttcgatac | 0.152 |
| Tg36 | 11.080 | tgttttaaactgaagatgtgtaaaatg | gaatgagcaagttaaacagtaagg | 0.145 |
| SL10027i | 11.098 | ctaccaggagcctgaagagc | ccattagagccaagacgctc | 0.456 |
| TG180 | 12.000 | tctcagtggactaaggggtca | gcatggaacaccatcatcaa | 0.216 |
| TG68 | 12.009 | tgcactaagcatctcgcatt | tttcatgtcaaggggattga | 0.304 |
| SL10953I | 12.029 | ctgtctctcgcttttctcctg | acggaacacaccctaagtgc | 0.184 |
| TG360 | 12.038 | ccccagaacacctctccata | tttcccgattttgttcctga | 0.264 |
| C2_At5g42740 | 12.055 | agcaccatttgagaaaaatatacctg | atccaaggaatgaaacattccacac | 0.370 |
| leoh301 | 12.066 | tgctgttttgtttggctcac | tgttcatatctttgatggcatgt | 0.312 |
| CosOH1 | 12.070 | tgcatacacttggtcatgacttc | ggctatagcatgcgttggtt | 0.909 |
| LEOH 275 | 12.075 | tcctctgaaaacaacttcacga | agtgtgagcctcaaattcca | 0.588 |
| PtiB | 12.088 | gcccctgatatggcagcacgtc | caaggcagcaactgcagccatc | 0.904 |

[x] approximate position obtained from Dynze, *et al.*, 2007.

Table 3-2. Detection of associated molecular markers in 354 $F_2$ individuals for chromosome 10 & 11 and for a subset of 135 $F_2$ plants for chromosome 4, 10 and 11 through single marker interval analysis by fitting data to simple linear regression model.

| Chromosome | Marker | b0 | b1 | -2ln(L0/L1)[z] | F-value | *p*-value |
|---|---|---|---|---|---|---|
| 354 $F_2$ plants | | | | | | |
| 10 | SL10105i | 3.339 | -0.177 | 5.376 | 5.385 | 0.021* |
| 11 | T0408-1,2 | 3.342 | 0.152 | 4.058 | 4.059 | 0.045* |
| 135 $F_2$ plants (subset of 354) | | | | | | |
| 4 | SSR296 | 2.528 | -0.082 | 0.16 | 0.158 | 0.692 |
| 10 | SL10105i | 2.509 | -0.442 | 5.102 | 5.122 | 0.025* |
| 11 | T0408-1,2 | 2.503 | 0.385 | 4.426 | 4.433 | 0.037* |

[z] Likelihood ratio test statistic.

Table 3-3. Parents and $F_2$ plant survival percentage as per combination of alleles on chromosome 10 and 11. Plant numbers are in parenthesis.

| SL10105i[v] | T0408-1,2[w] | Resistant | Susceptible |
|---|---|---|---|
| PI 126932 | | | |
| SP[x] | SP | 91.60(22) | 8.4(2) |
| | | | |
| Fla. 7776 | | | |
| SL[y] | SL | 12.5(3) | 87.5(21) |
| | | | |
| F2 generation | | | |
| SP | SP | 25.00(2) | 75.00(6) |
| | Het | 63.64(7) | 36.36(4) |
| | SL | 77.78(7) | 22.22(2) |
| | | | |
| Het[z] | SP | 23.53(4) | 76.47(13) |
| | Het | 79.07(34) | 20.93(9) |
| | SL | 37.50(6) | 62.50(10) |
| | | | |
| SL | SP | 28.57(4) | 71.43(10) |
| | Het | 23.08(3) | 76.92(10) |
| | SL | 50.00(3) | 50.00(3) |

[v] Marker on chromosome 10.
[w] Marker on chromosome 11.
[x] Homozygous for PI 126932 allele.
[y] Heterozygous for both alleles.
[z] Homozygous for Fla. 7776 allele.

Table 3-4. $F_3$ plant survival percentage as per combination of alleles on chromosome 10 and 11. Plant numbers are in parenthesis.

| SL10105i[v] | T0408-1,2[w] | Resistant | Susceptible |
|---|---|---|---|
| SP[x] | SP | 28.57(2) | 71.43(5) |
| | Het | 50.00(7) | 50.00(7) |
| | SL | 88.89(8) | 11.11(1) |
| Het[y] | SP | 33.33(2) | 66.67(4) |
| | Het | 63.64(7) | 36.36(4) |
| | SL | 50.00(5) | 50.00(5) |
| SL[z] | SP | 13.33(2) | 86.67(13) |
| | Het | 18.18(2) | 81.82(9) |
| | SL | 40.00(2) | 60.00(3) |

[v] Marker on chromosome 10.
[w] Marker on chromosome 11.
[x] Homozygous for PI 126932 allele.
[y] Heterozygous for both alleles.
[z] Homozygous for Fla. 7776 allele.

Table 3-5. $BC_1$ plant survival percentage as per combination of alleles on chromosome 10 and 11. Plant numbers are in parenthesis.

| SL10105i[w] | T0408-1,2[x] | Resistant | Susceptible |
|---|---|---|---|
| Het[y] | Het | 83.3(15) | 16.6(3) |
| | SL | 75(12) | 25(4) |
| SL[z] | Het | 33.3(36) | 66.66(12) |
| | SL | 58.3(7) | 41.6(5) |

[w] Marker on chromosome 10.
[x] Marker on chromosome 11.
[y] Heterozygous for both alleles.
[z] Homozygous for Fla. 7776 allele.

Table 3-6. Detection of associated molecular markers in 64 $BC_1$ individuals through single marker interval analysis by fitting data to simple linear regression model.

| Chromosome | Marker | b0 | b1 | -2ln(L0/L1)[z] | F-value | *p*-value |
|---|---|---|---|---|---|---|
| 10 | SL10105i | 2.412 | -0.605 | 5.054 | 5.094 | 0.028* |
| 11 | T0408-1,2 | 2.917 | 0.506 | 3.451 | 3.435 | 0.069 |

[z] Likelihood ratio test statistic

# CHAPTER 4
# SUMMARY AND CONCLUSIONS

Southern blight of tomato (*Solanum lycopersicum* L.) caused by *Sclerotium rolfsii* Sacc. is a minor soil borne disease in Florida but the economic losses caused by this fungus could increase after the regulatory phase out of Methyl Bromide which is the preferred method of control. The best alternative control of this disease is the deployment of genetically resistant cultivars which will not only be economical but also beneficial for low-input and organic crop production systems. This study confirmed that the previously identified (Mohr, 1955) *S. pimpinellifolium* accession (PI 126932) was an effective source of resistance against a Florida isolate of *S*. *rolfsii* and determined the genetic components of southern blight resistance from that source. This information will be used to incorporate resistance into breeding lines through marker assisted selection.

Earlier work showed that isolates of *S. rolfsii* could differ in their virulence (Sharma *et al*., 2002; Flores-Moctezume *et al*., 2006; Shukla and Pandey, 2008). So the first step was to determine if there was any difference in the level of resistance among the reported sources of resistance against several diverse isolates of *S. rolfsii*. This would identify the most effective source of resistance for genetic mapping. Two resistant sources (PI 126932 and breeding line 5913M), and a susceptible inbred line (Fla. 7776) were individually inoculated with an isolate of *S. rolfsii* collected from tomato (GCT-1), peanut (WM 609) and sweet potato (DF/LA SR1), obtained from Florida, Georgia and Louisiana respectively.

Results showed that PI 126932 provided resistance against all three isolates of *S. rolfsii* used in the study, while breeding line 5913M was only resistant against isolate WM609 from peanut and DF/LA SR1 from sweet potato but not against GCT-1 from

tomato. Fla. 7776 was susceptible to all three isolates. Significant differences were observed between different genotypes of tomato and between the three isolates of *S. rolfsii*. Significant interaction between the tomato lines and the *S. rolfsii* strains was also noted. This showed that the resistance in tomato could depend on the interaction between the tomato genotype and southern blight strain.

For genetic studies of southern blight resistance, PI 126932 and Fla. 7776 were selected as resistant and susceptible parents respectively. Crosses were made between the parents and several generations were created. Both parents and $F_1$ and $F_2$ generations were artificially inoculated with southern blight strain GCT-1. Results indicated that the genes contributing to resistance in PI 126932 could have incomplete penetrance. However, such assumption needs to be confirmed by further tests as in the field trial (appendix D) it seemed that high inoculum pressure and favorable climatic condition for pathogen could be a possible cause for death of plants of resistant lines. The distributions of individual phenotypes in the $F_1$ and $F_2$ generations suggested that the inheritance of this trait in tomato involved multiple genes. Genetic mapping experiments revealed two loci affecting resistance. Initially three loci were detected in a selective genotyping study using the $F_2$ segregating generation inoculated with the GCT-1 isolate and genotyped with 102 co-dominant molecular markers. One locus was excluded in a follow-up experiment with a larger population. The other two loci L1 (chromosome 10) and L2 (chromosome 11) were confirmed in a larger $F_2$ population without selective genotyping and $F_3$ and $BC_1$ populations. Locus L1 was found to be contributing resistance while effect of L2 was opposite to that of L1. Paradoxically, the highest numbers of resistant plants in $F_2$, $F_3$ and $BC_1$ generation were found to be

heterozygous for both loci. One explanation involves both an overdominance and epistasis effect. In the Fla. 7776 genetic background, locus L1 alone was found to provide a good resistance level. Data indicated epistasis effect for locus L1, suggesting that this gene might be interacting with some other genes along with L2. If the resistance requires an interaction between genes then such a condition would greatly affect the transfer of resistance genes through backcrossing. However, evidence from $BC_1$ generation suggested that a single gene (L1) from PI 126932 was sufficient in the Fla. 7776 background for providing resistance and by use of the molecular backcross method it could be transferred to cultivated lines to increase their resistance. Heterozygous plants for locus L1 were also found to be resistant (dominant inheritance), thus L1 can be used for breeding southern blight resistance into $F_1$ hybrids because introgression of this locus is only needed in one parent.

The mechanisms underlying the resistance conferred by this gene remain to be resolved. *S. rolfsii* is a necrotrophic pathogen which seems to be relying on three prime mechanisms for overcoming its host plants, i.e. the ability to kill host cells, decompose plant tissue and counteract plant defense responses (Glazebrook, 2005). In order to kill host cells, the fungus is able to produce certain phytotoxic metabolites, such as oxalic acid (OA) and polygalacturonase (Bateman and Beer, 1965). OA induces Programmed Cell Death (PCD) in the host plants (Errakhi *et al*., 2008). PCD is a part of plant life cycle and helps in plant tissue development (Kim *et al.,* 2008). Hypersensitive response is a form of PCD which act as a defense mechanism during certain pathogens attack. However, under attack from certain necrotrophic fungus like *S. rolfsii*, *Sclerotinia sclerotiorum* PCD is beneficial to the pathogen as compared to the host (Kim *et al.,*

2008). While high concentration of OA causes PCD, at lower concentration OA induces defense related gene expression resulting in resistance against the proliferation of *S. rolfsii* in *Arabidopsis thaliana* (Lehner, 2008). It could be possible that the genes at the detected loci might be somehow degrading the effect of OA, but this remains to be determined. The second trait employed by this fungus in subduing its host is decomposing host tissue through plant cell wall degrading enzymes like polygalacturonase. OA increases the rate by which polygalacturonase hydrolyze pectates in the middle lamella by reducing the pH of host tissue to a more favorable pH range for enzyme action and by combining oxalate with the calcium ions in the calcium pectates of the host cell wall (Bateman and Beer, 1965). It could be possible that the genes are inhibiting the chelating of calcium ions in the calcium pectates in the middle lamelle thereby inhibiting the effect of polygalacturonase and rendering resistance to the host. Third principle on which this fungus relies to overcome host plant is its ability to counteract the action of host defense mechanism. *S. rolfsii* is capable of doing this by suppressing the oxidative burst, which is one of the initial plant defense mechanism (Kim *et al,* 2008). The loci identified in this study might confer a better oxidative burst response leading to efficient production of hydrogen peroxide ($H_2O_2$) which acts as antimicrobial agent. Future studies should identify the gene(s) and unravel the mechanisms for the higher resistance levels by the detected genes.

Another possible mechanism for resistance could be due to phellem cells that form a protective barrier eventually preventing entry of fungus into the host stem (Mohr and Watkins, 1959). In such case using tomato rootstock possessing continuous phellem cells layer could help in controlling this disease. Grafting trials were conducted to test if

resistance could be achieved by using PI 126932 as a rootstock but due to unfavorable condition no results were obtained (appendix E).

Further research is required to gain better understanding of southern blight genetics in tomato. The influence of parameters like environmental effect, incomplete penetrance, and fungus strain effect points to the need for large segregating populations and progeny testing in $F_3$ families to maximize the ability to detect resistance loci and to understand their effects. It would also be helpful to evaluate different crosses involving various sources of resistant and susceptible lines. Based on the results of earlier work and the current study it is likely that resistance from PI 126932 will be effective against southern blight under field conditions. Current studies were carried out in greenhouse conditions. Field trials were conducted but due to unfavorable conditions no valid conclusions were derived (appendix D). So, the resistance needs to be tested in field conditions and against various isolates of *S. rolfsii*. Developing polymorphic markers in future for the chromosomal regions which were not covered in this study could perhaps help in detecting additional genes with weak effects.

The phase-out of Methyl Bromide (MeBr) has forced researchers to come up with new alternatives for controlling soil-borne diseases. Minor soil borne diseases like southern blight which were effectively controlled by MeBr could now become major problems to the Florida tomato industry. This study was directed towards finding genes which could help in establishing southern blight resistant tomato lines. For the first time we were able to map genes contributing to the resistance against southern blight in tomato which are now being used to transfer resistance to elite commercial tomato lines.

# APPENDIX A
# PEDIGREES

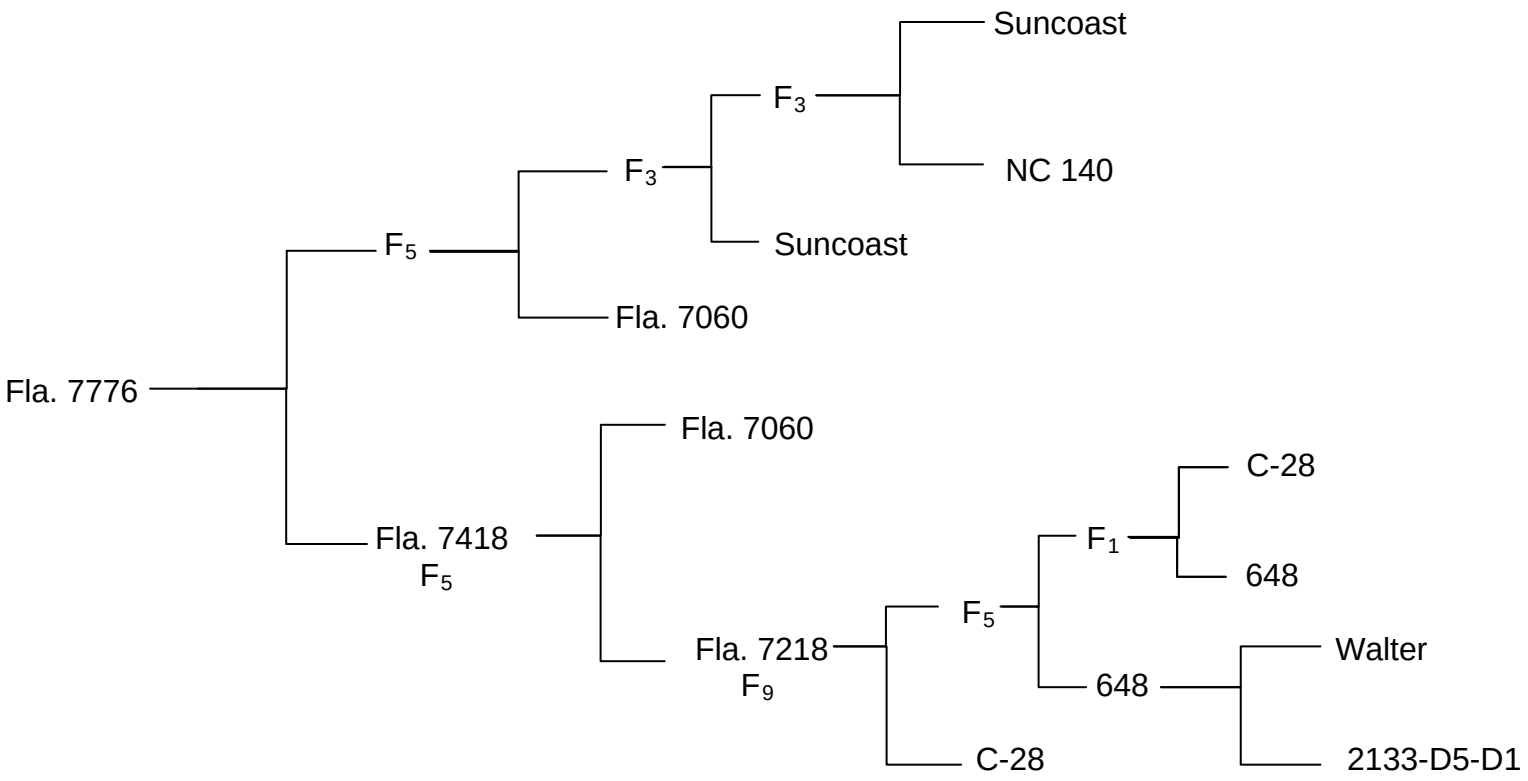


Figure A-1. Pedigree of Fla. 7776.

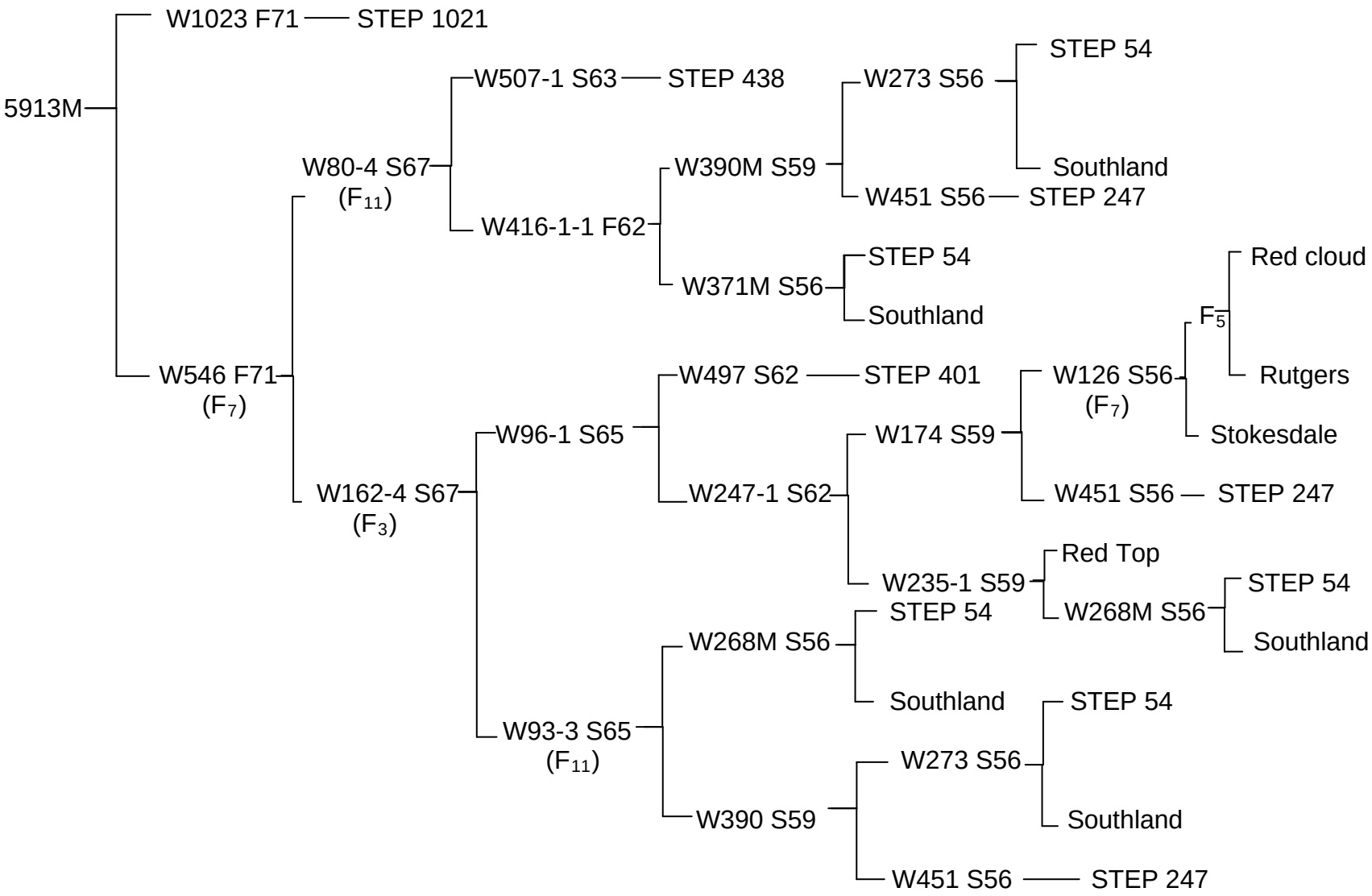


Figure A-2. Pedigree of 5913M.

# APPENDIX B
# MOLECULAR MARKER TECHNICAL INFORMATION

Table B-1. Technical information for markers polymorphic between Fla. 7776 and PI 126932.

| Marker | Location | Marker type | Restriction Enzyme | Annealing Temp. | Amplicon size | Detection | Reference |
|---|---|---|---|---|---|---|---|
| CosOH47 | 1.010 | caps | *Bst UI* | 54 | 226-150 | 2% agarose | SGN |
| LEOH36 | 1.017 | caps | *Bcl I* | 56 | 1300-1128 | 1% agarose | Yang, 2004 |
| C2_At5g18580 | 1.035 | caps | *Hpy CH41V* | 49 | 929-1100 | 2% agarose | SGN |
| SSR95 | 1.043 | ssr | N/A | 45 | 212-224 | 6.5% acrylamide | SGN |
| SSR316 | 1.053 | ssr | N/A | 45 | 235-251 | 6.5% acrylamide | SGN |
| SL20268i | 1.061 | indel | N/A | 45 | 234-248 | 6.5% acrylamide | Deynze, 2007 |
| SL10975i | 1.070 | scar | N/A | 55 | 161-151 | 6.5% acrylamide | Deynze, 2007 |
| LEVCOH11 | 1.085 | caps | *Mnl I* | 54 | 149-195 | 4% agarose | Yang, 2005 |
| C2_At3g04710 | 1.095 | caps | *Hinc II* | 55 | 900-1100 | 2% agarose | SGN |
| SSR42 | 1.107 | ssr | N/A | 45 | 188-192 | 6.5% acrylamide | SGN |
| SL10126i | 1.137 | indel | N/A | 45 | 213-219 | 6.5% acrylamide | Deynze, 2007 |

Table B-1. Continued

| Marker | Location | Marker type | Restriction Enzyme | Annealing Temp. | Amplicon size | Detection | Reference |
|---|---|---|---|---|---|---|---|
| TOM11 | 2.013 | ssr | N/A | 45 | 183-187 | 6.5% acrylamide | SGN |
| SL10682i | 2.034 | scar | N/A | 45 | 182-176 | 6.5% acrylamide | Deynze, 2007 |
| SSR96 | 2.043 | ssr | N/A | 50 | 199-209-221 | 6.5% acrylamide | SGN |
| SSR5 | 2.050 | ssr | N/A | 50 | 196-193-181 | 6.5% acrylamide | SGN |
| LEOH348 | 2.064 | caps | *Hpy CH41V* | 52 | 100-184 | 2% agarose | Yang, 2005 |
| LEOH113 | 2.075 | snp | *Nla III* | 52 | 154-211 | 4% agarose | Yang, 2004 |
| C2_At5g66090 | 2.083 | caps | *Hpy CH4III* | 55 | 300-450 | 2% agarose | SGN |
| LEOH174 | 2.096 | indel | N/A | 52 | 221-146 | 4% agarose | Yang, 2005 |

Table B-1. Continued

| Marker | Location | Marker type | Restriction Enzyme | Annealing Temp. | Amplicon size | Detection | Reference |
|---|---|---|---|---|---|---|---|
| TG525 | 3.014 | caps | *Tsp509I* | 53 | 214-230 | 2% agarose | SGN |
| LEOH124i | 3.021 | indel | N/A | 52 | 110-208 | 6.5% acrylamide | Yang, 2004 |
| SL10480i | 3.038 | indel | N/A | 45 | 100-150 | 2% agarose | Deynze, 2007 |
| LEOH223 | 3.040 | caps | *Mse I* | 52 | 177-212 | 4% agarose | Yang, 2005 |
| C2_At1g02140 | 3.054 | caps | *Hha I* | 50 | 700-1000-1550 | 2% agarose | SGN |
| FEY | 3.064 | caps | *Bst Ui* | 55 | 667-800 | 2% agarose | SGN |
| C2_At5g60160 | 3.068 | caps | *Hinf I* | 54 | 450-500 | 4% agarose | SGN |
| C2_At5g52820 | 3.070 | caps | *HypCH4IV* | 55 | 750-850 | 2% agarose | SGN |
| C2_At1g61620 | 3.087 | caps | *Taq I* | 54 | 600-900 | 2% agarose | SGN |
| LEOH127 | 3.098 | caps | *Hinc II* | 52 | 177-244 | 2% agarose | Yang, 2004 |

Table B-1. Continued

| Marker | Location | Marker type | Restriction Enzyme | Annealing Temp. | Amplicon size | Detection | Reference |
|---|---|---|---|---|---|---|---|
| SSR296 | 4.010 | ssr | N/A | 45 | 185-200 | 6.5% acrylamide | SGN |
| SL10255i | 4.025 | indel | N/A | 45 | 160-150 | 6.5% acrylamide | Deynze, 2007 |
| C2_At3g17040 | 4.032 | caps | *Dde I* | 55 | 200-250-550 | 2% agarose | SGN |
| SSR603 | 4.045 | ssr | N/A | 45 | 251-179 | 6.5% acrylamide | SGN |
| SSR306 | 4.053 | ssr | N/A | 45 | 255-270 | 6.5% acrylamide | SGN |
| C2_At1g71810 | 4.072 | caps | *Bst UI* | 57 | 450-800-1100 | 2% agarose | SGN |
| CT194 | 4.079 | ssr | N/A | 45 | 174-171 | 6.5% acrylamide | SGN |
| SL00030 | 4.087 | snp | *Ase I* | 51 | 233-163-70 | 4% Metaphor | SolCAP[X] |
| SSR146 | 4.107 | ssr | N/A | 45 | 234-238 | 6.5% acrylamide | SGN |

[X] http://solcap.msu.edu/tomato_snp_survey.shtml. [Accessed: January, 2010].

Table B-1. Continued

| Marker | Location | Marker type | Restriction Enzyme | Annealing Temp. | Amplicon size | Detection | Reference |
|---|---|---|---|---|---|---|---|
| TG441 | 5.008 | caps | *TaqI* | 53 | 350-280 | 2% agarose | SGN |
| P11M6 | 5.015 | caps | *Taq I* | 50 | 300-330 | 4% agarose | Hutton, 2008 |
| Bs4 | 5.022 | caps | *Dpn II* | 55 | 600-650 | 2% agarose | SGN |
| CT93 | 5.030 | caps | *Alu I* | 54 | 300-325 | 2% agarose | Hutton, 2008 |
| SL20210i | 5.046 | scar | N/A | 52 | 170-250 | 2% agarose | Deynze, 2007 |
| C2_At1g14000 | 5.060 | caps | *Spe I* | 55 | 900-1000 | 2% agarose | SGN |
| TOM49 | 5.068 | ssr | N/A | 45 | 223-190 | 6.5% acrylamide | Suliman,2002 |
| CosOH73 | 5.082 | caps | *Alu I* | 56 | 69-123 | 2% agarose | tomatomap.net |
| SSR162 | 5.090 | ssr | N/A | 50 | 260-264 | 6.5% acrylamide | SGN |

Table B-1. Continued

| Marker | Location | Marker type | Restriction Enzyme | Annealing Temp. | Amplicon size | Detection | Reference |
|---|---|---|---|---|---|---|---|
| SL10328i | 6.005 | scar | N/A | 45 | 234-246 | 6.5% acrylamide | Deynze, 2007 |
| T1456 | 6.010 | caps | *Rsa I* | 56 | 500-650 | 2% agarose | Ji *et al.*2007 |
| p55P11 | 6.020 | caps | *DdeI* | 54 | 300-400 | 2% agarose | SGN |
| TG590f2R2 | 6.029 | caps | *HpyCH4III* | 55 | 250-350-525 | 2% agarose | Hutton, 2008 |
| P6-25F2R5 | 6.025 | caps | *Taq I* | 55 | 200-400 | 2% agarose | Hutton, 2008 |
| T0834-Fla,R2 | 6.032 | scar | N/A | 55 | 550-660 | 2% agarose | Hutton, 2008 |
| C2_At1g44760 | 6.044 | caps | *Nsi I* | 55 | 400-700-900 | 2% agarose | SGN |
| SP | 6.068 | caps | *Bst NI* | 56 | 350-370 | 4% agarose | SGN |
| LEOH112 | 6.078 | caps | *HpyCH4IV* | 52 | 240-300 | 4% agarose | Yang, 2004 |
| SSR350 | 6.100 | ssr | N/A | 45 | 149-267-269 | 6.5% acrylamide | SGN |

Table B-1. Continued

| Marker | Location | Marker type | Restriction Enzyme | Annealing Temp. | Amplicon size | Detection | Reference |
|---|---|---|---|---|---|---|---|
| C2_At5g20180 | 7.006 | caps | *Taq I* | 55 | 700-800-1300 | 2% agarose | SGN |
| C2_At1g19140 | 7.024 | scar | N/A | 55 | 855-1100 | 2% agarose | SGN |
| SSR276 | 7.033 | ssr | N/A | 52 | 150-177 | 4% agarose | SGN |
| TG217 | 7.043 | caps | *HpyCH4IV* | 55 | 450-500 | 4% agarose | SGN |
| TG174 | 7.052 | caps | *Hha I* | 55 | 500-1200 | 2% agarose | SGN |
| TG216-1 | 7.062 | caps | *Bsl I* | 55 | 300-350 | 2% agarose | Hutton, 2008 |
| C2_At1g56130 | 7.085 | caps | *RsaI* | 55 | 400-300 | 2% agarose | SGN |

Table B-1. Continued

| Marker | Location | Marker type | Restriction Enzyme | Annealing Temp. | Amplicon size | Detection | Reference |
|---|---|---|---|---|---|---|---|
| C2_At5g46630 | 8.000 | CAPS | *Hpy CH4III* | 56 | 425-700 | 2% agarose | SGN |
| LEOH147 | 8.020 | caps | *Tsp45i* | 52 | 117-185 | 2% agarose | Yang, 2004 |
| C2_At2g26830 | 8.030 | CAPS | *Hpy CH4III* | 50 | 550-950 | 2% agarose | SGN |
| TG302 | 8.038 | CAPS | *Alu I* | 55 | 450-700 | 2% agarose | SGN |
| SSR38 | 8.053 | SSR | N/A | 50 | 237-240 | 6.5% acrylamide | SGN |
| TG294 | 8.083 | CAPS | *Alu I* | 50 | 800-550 | 2% agarose | SGN |

Table B-1. Continued

| Marker | Location | Marker type | Restriction Enzyme | Annealing Temp. | Amplicon size | Detection | Reference |
|---|---|---|---|---|---|---|---|
| C09HBa0203J14.1 | 9.023 | caps | *Tsp 509I* | 53 | 300-380 | 3% agarose | Hutton, 2008 |
| SSR70 | 9.031 | ssr | N/A | 50 | 115-105 | 6.5% acrylamide | SGN |
| LEOH144 | 9.058 | caps | *Fok I* | 52 | 152-225 | 2% agarose | Yang, 2004 |
| LEOH170 | 9.074 | indel | N/A | 52 | 212-384 | 2% agarose | Yang, 2005 |
| SSR333 | 9.100 | ssr | N/A | 50 | 199-201 | 6.5% acrylamide | SGN |

Table B-1. Continued

| Marker | Location | Marker type | Restriction Enzyme | Annealing Temp. | Amplicon size | Detection | Reference |
|---|---|---|---|---|---|---|---|
| C2_At3g21610 | 10.000 | caps | *Dpn II* | 55 | 500-750 | 2% agarose | SGN |
| C2_At5g60990 | 10.014 | caps | *Hinf I* | 55 | 900-1100 | 2% agarose | SGN |
| SL10105i | 10.030 | scar | N/A | 45 | 203-235 | 6.5% acrylamide | Dynze, 2007 |
| LEVCOH15 | 10.037 | scar | N/A | 52 | 178-188 | 6.5% acrylamide | Yang, 2005 |
| SL10419i | 10.043 | scar | N/A | 45 | 105-124 | 2% agarose | Dynze, 2007 |
| C2_At3g58470 | 10.061 | caps | *Tsp 5091* | 50 | 320-280 | 2% agarose | SGN |
| T1682 | 10.066 | caps | *Hinf I* | 54 | 220-300 | 2% agarose | Hutton, 2008 |
| TG403(dCAPS)R | 10.082 | dcaps | *Hpy188I* | 55 | 215-235 | 4% agarose | Hutton[y] |

[y] Personal Communication.

Table B-1. Continued

| Marker | Location | Marker type | Restriction Enzyme | Annealing Temp. | Amplicon size | Detection | Reference |
|---|---|---|---|---|---|---|---|
| SL10683i | 11.000 | indel | N/A | 45 | 165-171 | 6.5% acrylamide | Dynze, 2007 |
| TG497 | 11.004 | caps | *Taq I* | 52 | 550-1200 | 2% agarose | SGN |
| SSR80 | 11.017 | ssr | N/A | 45 | 164-167 | 4% agarose | SGN |
| T0408-1,2 | 11.026 | caps | *Mnl I* | 55 | 350-520 | 2% agarose | Hutton, 2008 |
| SSR76 | 11.046 | ssr | N/A | 45 | 150-160 | 2% agarose | SGN |
| C2_At4g10050 | 11.054 | caps | *BstN I* | 56 | 139-170 | 2% agarose | SGN |
| SL10737i | 11.060 | scar | N/A | 45 | 163-176 | 6.5% acrylamide | Dynze, 2007 |
| cLET-24-J2 | 11.073 | caps | *Hpy CH4III* | 55 | 394-450 | 4% agarose | Hutton, 2008 |
| TG36 | 11.080 | ssr | N/A | 45 | 162-172 | 6.5% acrylamide | SGN |
| SL10027i | 11.098 | indel | N/A | 45 | 171-180 | 6.5% acrylamide | Dynze, 2007 |

Table B-1. Continued

| Marker | Location | Marker type | Restriction Enzyme | Annealing Temp. | Amplicon size | Detection | Reference |
|---|---|---|---|---|---|---|---|
| TG180 | 12.000 | caps | *DraI* | 55 | 270-490 | 2% agarose | SGN |
| TG68 | 12.009 | caps | *EcoRV* | 52 | 200-300 | 2% agarose | SGN |
| SL10953i | 12.029 | indel | N/A | 45 | 219-231 | 6.5% acrylamide | Deynze, 2007 |
| TG360 | 12.038 | caps | *Apo I* | 55 | 500-650 | 2% agarose | Hutton, 2008 |
| C2_At5g42740 | 12.055 | caps | *Dde I* | 55 | 700-600 | 2% agarose | SGN |
| LEOH301 | 12.066 | scar | N/A | 52 | 164-185 | 4% agarose | Yang, 2005 |
| CosOH1 | 12.070 | caps | *Tsp RI* | 54 | 510-380 | 2% agarose | SGN |
| LEOH 275 | 12.075 | snp | *MseI* | 52 | 88-144 | 2% agarose | Yang, 2005 |
| PtiB | 12.088 | caps | *MnI* | 56 | 500-600 | 2% agarose | SGN |

# APPENDIX C
# ADDITIONAL MOLECULAR MARKER INFORMATION

Table C-1. Marker classification based on polymorphism and dominance between PI 126932 and Fla. 7776.

| Markers | Approx position | Polymorphic | Dominant | Co-dominant |
|---|---|---|---|---|
| CosOH47 | 1.010 | Yes | No | Yes |
| SSR 34 | 1.013 | No | - | - |
| SL10075 | 1.015 | No | - | - |
| LEOH36 | 1.017 | Yes | No | Yes |
| TG58 | 1.017 | No | - | - |
| TG236 | 1.019 | No | - | - |
| C01HBa0003D15.1 | 1.029 | Yes | Yes | No |
| SSR 266 | 1.033 | No | - | - |
| SSR192 | 1.033 | No | - | - |
| C2_At5g18580 | 1.035 | Yes | No | Yes |
| SSR 105 | 1.040 | No | - | - |
| TOM202 | 1.041 | Yes | No | Yes |
| SSR95 | 1.043 | Yes | No | Yes |
| SSR 316 | 1.053 | Yes | No | Yes |
| SL20134i | 1.060 | Yes | No | Yes |
| SL20268i | 1.061 | Yes | No | Yes |
| SSR 134 | 1.064 | Yes | No | Yes |
| SL10975i | 1.070 | Yes | No | Yes |
| SL20116 | 1.078 | No | - | - |
| SL 10945 | 1.084 | Yes | No | Yes |
| LEVCOH11 | 1.085 | Yes | No | Yes |
| CT68 | 1.086 | No | - | - |
| SSR9 | 1.093 | No | - | - |
| C2_At3g04710 | 1.095 | Yes | No | Yes |
| U237757 | 1.102 | Yes | Yes | No |
| SSR42 | 1.107 | Yes | No | Yes |
| t0664 | 1.111 | No | - | - |
| C2_At1g02560 | 1.116 | Yes | Yes | No |
| SSR37 | 1.118 | No | - | - |
| TG255 | 1.125 | No | - | - |
| SL10126i | 1.137 | Yes | No | Yes |
| SSR595 | 1.147 | No | - | - |
| C2_At2g15890 | 1.150 | No | - | - |

Table C-1. Continued

| Markers | Approx position | Polymorphic | Dominant | Co-dominant |
|---|---|---|---|---|
| LEOH342 | 2.000 | Yes | Yes | No |
| SL10351 | 2.011 | No | - | - |
| TOM11 | 2.013 | Yes | No | Yes |
| CT205 | 2.023 | No | - | - |
| SL10682i | 2.034 | Yes | No | Yes |
| SSR96 | 2.043 | Yes | No | Yes |
| 120k | 2.045 | No | - | - |
| SSR5 | 2.050 | Yes | No | Yes |
| SL10279I | 2.060 | Yes | Yes | No |
| LEOH348 | 2.064 | Yes | No | Yes |
| Ovate | 2.073 | No | - | - |
| LEOH113 | 2.075 | Yes | No | Yes |
| TG337 | 2.082 | No | - | - |
| C2_At5g66090 | 2.083 | Yes | No | Yes |
| TG537 | 2.089 | No | - | - |
| TG91 | 2.090 | No | - | - |
| LEOH174 | 2.096 | Yes | No | Yes |
| LEOH319 | 2.097 | Yes | Yes | No |
| TG151 | 2.098 | No | - | - |
| TG154 | 2.105 | No | - | - |
| TG 114 | 3.000 | No | - | - |
| SL 10690i | 3.003 | Yes | Yes | No |
| TG130 | 3.006 | No | - | - |
| SL20182i | 3.013 | Yes | No | Yes |
| TG525 | 3.014 | Yes | No | Yes |
| LEOH124i | 3.021 | Yes | No | Yes |
| cbf | 3.029 | No | - | - |
| SL10480i | 3.038 | Yes | No | Yes |
| LEOH223 | 3.040 | Yes | No | Yes |
| SL20195 | 3.040 | Yes | No | Yes |
| t1388 | 3.047 | Yes | Yes | No |
| SL 10736 | 3.047 | Yes | No | Yes |
| SL20037 | 3.049 | Yes | No | Yes |

Table C-1 Continued

| Markers | Approx position | Polymorphic | Dominant | Co-dominant |
|---|---|---|---|---|
| SSR111 | 3.053 | No | - | - |
| C2_At1g02140 | 3.054 | Yes | No | Yes |
| FEY | 3.064 | Yes | No | Yes |
| C2_At5g60160 | 3.068 | Yes | No | Yes |
| C2_At5g52820 | 3.070 | Yes | No | Yes |
| t1659 | 3.083 | No | - | - |
| C2_At1g61620 | 3.087 | Yes | No | Yes |
| TG134B5 | 3.094 | No | - | - |
| U146899 | 3.097 | Yes | Yes | No |
| LEOH127 | 3.098 | Yes | No | Yes |
| HERO | 4.007 | Yes | Yes | No |
| SSR296 | 4.010 | Yes | No | Yes |
| TG15-2 | 4.021 | Yes | Yes | No |
| SSR43 | 4.025 | Yes | No | Yes |
| SL10255i | 4.025 | Yes | No | Yes |
| C2_At3g17040 | 4.032 | Yes | No | Yes |
| TG483 | 4.037 | No | - | - |
| SSR603 | 4.045 | Yes | No | Yes |
| SSR310 | 4.053 | No | - | - |
| SSR306 | 4.053 | Yes | No | Yes |
| C2_At1g71810 | 4.072 | Yes | No | Yes |
| CT185 | 4.076 | Yes | No | Yes |
| CT194 | 4.079 | Yes | No | Yes |
| SL_00045 | 4.083 | No | - | - |
| SL_00027 | 4.084 | No | - | - |
| SL00030 | 4.087 | Yes | No | Yes |
| C2_At1g27530 | 4.088 | Yes | Yes | No |
| CT50 | 4.092 | No | - | - |
| TG500 | 4.093 | Yes | Yes | No |
| SSR214 | 4.095 | No | - | - |
| SL10184 | 4.106 | Yes | No | Yes |
| SL10888 | 4.107 | Yes | No | Yes |
| SSR146 | 4.107 | Yes | No | Yes |

Table C-1 Continued

| Markers | Approx position | Polymorphic | Dominant | Co-dominant |
|---|---|---|---|---|
| TG163 | 4.107 | No | - | - |
| t0998 | 5.000 | No | - | - |
| TG441 | 5.008 | Yes | No | Yes |
| SSR115 | 5.014 | No | - | - |
| P11M6 | 5.015 | Yes | No | Yes |
| Bs4 | 5.022 | Yes | No | Yes |
| CT93 | 5.030 | Yes | No | Yes |
| LEOH16.2 | 5.033 | No | - | - |
| TG96 | 5.038 | No | - | - |
| SL20210i | 5.046 | Yes | No | Yes |
| t0040 | 5.056 | No | - | - |
| C2_At1g14000 | 5.060 | Yes | No | Yes |
| LEOH192 | 5.060 | No | - | - |
| TOM49 | 5.068 | Yes | No | Yes |
| LEOH316 | 5.069 | No | - | - |
| CosOH73 | 5.082 | Yes | No | Yes |
| TG185 | 5.087 | No | - | - |
| SSR162 | 5.090 | Yes | No | Yes |
| SL10328i | 6.000 | Yes | No | Yes |
| T1456 | 6.010 | Yes | No | Yes |
| ct216 | 6.012 | No | - | - |
| SL10242i | 6.018 | Yes | No | Yes |
| p55P11 | 6.020 | Yes | No | Yes |
| SL10187425 | 6.024 | Yes | No | Yes |
| P6-25F2R5 | 6.025 | Yes | No | Yes |
| TG590f2R2 | 6.029 | Yes | No | Yes |
| T0834-Fla,R2 | 6.032 | Yes | No | Yes |
| SSR128 | 6.041 | No | - | - |
| C2_At1g44760 | 6.044 | Yes | No | Yes |
| C2_At1g71950 | 6.046 | No | - | - |
| TG356 | 6.050 | No | - | - |
| LEOH243 | 6.053 | No | - | - |
| TG435 | 6.058 | Yes | Yes | No |

Table C-1 Continued

| Markers | Approx position | Polymorphic | Dominant | Co-dominant |
|---|---|---|---|---|
| TG365 | 6.060 | No | - | - |
| SP | 6.068 | Yes | No | Yes |
| LEOH146 | 6.069 | No | - | - |
| SCBC792 | 6.075 | Yes | Yes | No |
| CT206 | 6.078 | Yes | No | Yes |
| LEOH112 | 6.078 | Yes | No | Yes |
| TG314 | 6.090 | No | - | - |
| SSR350 | 6.100 | Yes | No | Yes |
| SL20017 | 7.003 | Yes | Yes | No |
| C2_At5g20180 | 7.006 | Yes | No | Yes |
| SSR286 | 7.012 | No | - | - |
| C2_At2g26590 | 7.015 | Yes | Yes | No |
| C2_At2g29490 | 7.022 | Yes | No | Yes |
| C2_At1g19140 | 7.024 | Yes | No | Yes |
| LEOH104 | 7.026 | No | - | - |
| SSR276 | 7.033 | Yes | No | Yes |
| C2_At2g20860 | 7.043 | Yes | No | Yes |
| TG217 | 7.043 | Yes | No | Yes |
| LEOH221 | 7.050 | No | - | - |
| LEOH40 | 7.050 | No | - | - |
| TG174 | 7.052 | Yes | No | Yes |
| TG291 | 7.053 | No | - | - |
| TG216-1 | 7.062 | Yes | No | Yes |
| SSR45 | 7.069 | Yes | No | Yes |
| SL10039 | 7.073 | No | - | - |
| C2_At1g56130 | 7.085 | Yes | No | Yes |
| TG499 | 7.085 | No | - | - |
| C2_At1g55870 | 7.090 | No | - | - |
| TG424 | 7.093 | No | - | - |
| C2_At5g56130 | 7.108 | No | - | - |
| C2_At5g46630 | 8.002 | Yes | No | Yes |
| LEOH 70 | 8.004 | No | - | - |
| U221657 | 8.013 | Yes | Yes | No |

Table C-1 Continued

| Markers | Approx position | Polymorphic | Dominant | Co-dominant |
|---|---|---|---|---|
| TG 176 | 8.017 | No | - | - |
| LEOH147 | 8.020 | Yes | No | Yes |
| C2_At5g27390 | 8.021 | Yes | No | Yes |
| SSR327 | 8.022 | No | - | - |
| SL10044 | 8.028 | No | - | - |
| C2_At2g26830 | 8.030 | Yes | No | Yes |
| TG349 | 8.031 | No | - | - |
| TG302 | 8.038 | Yes | No | Yes |
| C2_At3g43540 | 8.041 | Yes | No | Yes |
| SSR335 | 8.044 | No | - | - |
| SSR38 | 8.053 | Yes | No | Yes |
| CT265 | 8.069 | No | - | - |
| C2_At4g11560 | 8.072 | No | - | - |
| C2_At5g41350 | 8.075 | No | - | - |
| C2_At1g63980 | 8.077 | No | - | - |
| TG294 | 8.083 | Yes | No | Yes |
| TG254 | 9.000 | No | - | - |
| TG18 | 9.009 | No | - | - |
| LEOH8.4 | 9.010 | No | - | - |
| C2_At2g37025 | 9.015 | Yes | Yes | No |
| SL10471 | 9.020 | No | - | - |
| C09HBa0203J14.1 | 9.023 | Yes | No | Yes |
| SSR70 | 9.031 | Yes | No | Yes |
| LEOH31.4 | 9.040 | No | - | - |
| SSR28 | 9.050 | No | - | - |
| LEOH144 | 9.058 | Yes | No | Yes |
| LEOH 117 | 9.072 | No | - | - |
| LEOH170 | 9.074 | Yes | No | Yes |
| TG348 | 9.077 | No | - | - |
| TG421 | 9.081 | No | - | - |
| C2_At3g23400 | 9.084 | No | - | - |
| SSR333 | 9.100 | Yes | No | Yes |
| SSR599 | 9.104 | No | - | - |

Table C-1 Continued

| Markers | Approx position | Polymorphic | Dominant | Co-dominant |
|---|---|---|---|---|
| C2_At3g21610 | 10.002 | Yes | No | Yes |
| TG122 | 10.006 | No | - | - |
| T0787 | 10.009 | Yes | Yes | No |
| SSR34 | 10.013 | No | - | - |
| C2_At5g60990 | 10.014 | Yes | No | Yes |
| SL10105i | 10.030 | Yes | No | Yes |
| SSR318 | 10.033 | Yes | No | Yes |
| LEVCOH15 | 10.037 | Yes | No | Yes |
| SL10419i | 10.043 | Yes | No | Yes |
| TG285 | 10.045 | Yes | Yes | No |
| SL10386i | 10.054 | No | - | - |
| C2_At3g58470 | 10.061 | Yes | No | Yes |
| T1682 | 10.066 | Yes | No | Yes |
| SSR74 | 10.074 | No | - | - |
| SSR223 | 10.079 | No | - | - |
| SL10807 | 10.082 | No | - | - |
| TG403(dCAPS)R | 10.082 | Yes | No | Yes |
| SSR479 | 10.086 | No | - | - |
| C2_At2g273011.069 | 11.000 | No | - | - |
| SL10683i | 11.000 | Yes | No | Yes |
| TG497 | 11.004 | Yes | No | Yes |
| SSR80 | 11.017 | Yes | No | Yes |
| T0408-1,2 | 11.026 | Yes | No | Yes |
| SL20244i | 11.036 | No | - | - |
| C2_At4g22260 | 11.037 | No | - | - |
| TG384 | 11.046 | No | - | - |
| SSR76 | 11.046 | Yes | No | Yes |
| SL10737i | 11.054 | Yes | No | Yes |
| SL10615 | 11.057 | No | - | - |
| C2_At4g10050 | 11.060 | Yes | No | Yes |
| TOM144 | 11.062 | Yes | No | Yes |
| C2_At3g54470 | 11.072 | Yes | No | Yes |
| cLET-24-J2 | 11.073 | Yes | No | Yes |

Table C-1 Continued

| Markers | Approx position | Polymorphic | Dominant | Co-dominant |
|---|---|---|---|---|
| TG46 | 11.076 | No | - | - |
| TG36 | 11.080 | Yes | No | Yes |
| TG393 | 11.088 | No | - | - |
| SL10027i | 11.098 | Yes | No | Yes |
| TG180 | 12.000 | Yes | No | Yes |
| SL10925i | 12.000 | Yes | No | Yes |
| TG68 | 12.009 | Yes | No | Yes |
| SL10953i | 12.029 | Yes | No | Yes |
| CT100 | 12.036 | Yes | Yes | No |
| TG360 | 12.038 | Yes | No | Yes |
| CT99 | 12.045 | Yes | Yes | No |
| TG565 | 12.048 | Yes | No | Yes |
| TG111 | 12.053 | No | - | - |
| C2_At5g42740 | 12.055 | Yes | No | Yes |
| LEOH66 | 12.062 | No | - | - |
| LEOH301 | 12.066 | Yes | No | Yes |
| CosOH1 | 12.070 | Yes | No | Yes |
| LEOH 275 | 12.075 | Yes | No | Yes |
| SL10796i | 12.076 | Yes | Yes | No |
| LEOH197 | 12.086 | Yes | Yes | No |
| PtiB | 12.088 | Yes | No | Yes |

Table C-2. Chi-square test for marker segregation distortion.

| Marker | Chromosome | Genotype | Observed plants | Expected plants | *p*-value |
|---|---|---|---|---|---|
| SL10105i | 10 | SL[x] | 101 | 99.75 | |
| | | H[y] | 198 | 199.5 | 0.98 |
| | | SP[z] | 100 | 99.75 | |
| T0408-1,2 | 11 | SL | 107 | 99.75 | |
| | | H | 191 | 199.5 | 0.63 |
| | | SP | 101 | 99.75 | |

[x] Homozygous for Fla. 7776 allele.
[y] Heterozygous for both alleles.
[z] Homozygous for PI 126932 allele.

# APPENDIX D
# FIELD TRIAL EXPERIMENT

Resistance level of a particular plant genotype may vary between field and greenhouse conditions. Ajit *et al*., (2003) showed that the transgenic wheat lines earlier reported to be resistant against *Fusarium graminearum* under greenhouse conditions were found to be susceptible during field trials. Variation in resistance between field and greenhouse conditions could greatly affect the breeding of southern blight resistance into commercial tomato lines grown in fields. So, it was necessary to confirm that the level of southern blight resistance in various tomato genotypes under field conditions was consistent with the results obtained in the greenhouse trials during this study.

In order to evaluate the response to southern blight disease in various resistant and susceptible sources in field condition a study was carried out in September 2009. Two different susceptible sources Fla. 7776 and Fla. 47 while three resistant sources PI 126932, 5913M and 5635M were selected for this study. $F_1$ obtained from the cross between PI 126932 and Fla. 7776 was also included. *Sclerotium rolfsii* isolate GCT-1 was used in this study since this isolate was endemic to the location (GCREC) where this trial was conducted. A randomized complete block design was used with 4 replications, with 10 plants for each replication. All plant materials were raised in 128-well speedling trays for 4 weeks before field transplanting. Inoculation was done in a similar way as described in chapter 2 when plants were 8 weeks old; however, 3 g of inoculum load was used instead of 2 g per plant. Disease score was rated on a visual scale of 0 to 4. Data was collected on $10^{th}$ and $20^{th}$ day after inoculation (DAI).

A significant difference in the disease severity score was observed between the tomato lines tested for both the dates (Table D-1). A significant difference was also

observed between the replications (Table D-1) at 10 DAI. Variation in-between the replications could possibly explain why the mean disease severity score was lower for Fla. 7776 as compared to PI 126932 at 10 DAI. Both the breeding lines 5913M and 5635M were found to be susceptible against GCT-1 isolate at 10 and 20 DAI. Based on the results from the data collected on 10 DAI the lowest disease severity was seen in $F_1$ plants (Table D-2). Also at 10 DAI the disease score for $F_1$ was not found to be significantly different from Fla.7776 but was different from PI 126932; however based on the results from the data collected on 20 DAI, a significant difference was found between $F_1$ and Fla. 7776 but not between $F_1$ and PI 126932 (Table D-2). Most of the plants in all the tomato lines at 20 DAI were found to be susceptible. A possible cause for such a high rate of death could be due to higher inoculum pressure which could have overwhelmed the resistance. It is also likely that environmental were highly favorable for the pathogen which enabled it to kill even the resistant lines. Unfortunately due to limited time the study was not repeated and hence no definite conclusions were derived from this study.

Table D-1. Two-way analysis of variance test for determining variation in disease severity scores in tomato lines PI 126932, 5913M, 5635M, Fla. 7776, Fla. 47 and $F_1$(Fla. 7776 x PI 126932) in field condition. GCT-1 isolate of *S. rolfsii* was used to inoculate the plants.

| Source of variance | F value | *P*- value[y] |
|---|---|---|
| 10 days after inoculation | | |
| Lines | 11.22 | <0.01* |
| Reps | 3.15 | 0.03* |
| 20 days after inoculation | | |
| Lines | 5.02 | <0.01* |
| Reps | 2.49 | 0.06 |

[y] *P*- value based on 0.05 significance level.

Table D-2. Bonferroni's t test for differentiating tomato lines based on disease severity scores for GCT-1 isolate.

| No. | Line | Mean | Bonferroni's grouping[x] | MSD[y] |
|---|---|---|---|---|
| 10 DAI | | | | |
| 1 | 5635M | 2.9 | A [z] | |
| 2 | 5913M | 2.75 | A | |
| 3 | Fla.47 | 2.45 | A B | 0.75 |
| 4 | PI 126932 | 2.31 | A B | |
| 5 | Fla. 7776 | 1.85 | B C | |
| 6 | $F_1$[w] | 1.45 | C | |
| 20 DAI | | | | |
| 3 | Fla. 47 | 4 | A | |
| 2 | 5913M | 3.95 | A | |
| 1 | 5635M | 3.95 | A B | 0.38 |
| 5 | Fla. 7776 | 3.87 | A B | |
| 4 | PI 126932 | 3.62 | B C | |
| 6 | $F_1$ | 3.48 | C | |

[w] $F_1$ derived from cross between PI 126932(♂) and Fla. 7776(♀).
[x] Critical 't' value = 2.96.
[y] Minimum Significant Difference.
[z] Means with the same letters are not significantly different.

# APPENDIX E
# SOUTHERN BLIGHT RESISTANCE THROUGH GRAFTING

Many tomato soil-borne diseases like *Fusarium oxysporum* f. sp.lycopersici. and *P. lycopersici*. etc have been found to be controlled by grafting susceptible varieties onto resistant rootstocks (Lee, 2003; Lee and Oda, 2003; Rivard and Louws, 2008). Hence, in order to test whether PI 126932 could provide resistance against southern blight if used as a rootstock, a pilot study was carried out in greenhouse and later studies were carried out in the field.

In order to obtained grafted plants for the greenhouse study, 14 plants of Fla. 7776(scion) were grafted to PI 126932(rootstock) and also 14 plants of PI 126932(scion) were grafted to Fla. 7776(rootstock). The 3-week-old plants were grafted and allowed to grow for 5 more weeks before inoculation. However, at the end only 3 plants with Fla. 7776 as rootstock and 8 plants with PI 126932 as rootstock were obtained. The study was still carried out by inoculating the grafted plants and 10 plants of both parents as controls in greenhouse using a completely random design. For field trials plant materials included PI 126932, Fla. 7776, PI 126932 grafted to PI 126932 below cotyledon, Fla. 7776 grafted to Fla. 7776 below cotyledon, PI 126932 grafted to Fla. 7776 below cotyledon, and PI 126932 grafted to Fla. 7776 above cotyledon. A randomized complete block design with four replications each including 10 plants per treatment was used. GCT-1 isolate was used for this study. Field trials were conducted in June 2009 and October 2009.

Due to extremely low number of grafted plants no statistical conclusions were derived from the greenhouse study (Table E-1). During the first field trial most of the plants died before inoculation due to accidental drift of herbicide from neighboring area.

Trial was repeated in October 2009 but due to sudden drop in temperature the inoculation was not successful and no data were obtained. Further studies were not conducted due to lack of time.

Table E-1. Number of grafted and parental lines plants found to be resistant and susceptible under greenhouse condition.

| | Susceptible | Resistant | Total |
|---|---|---|---|
| F>P[x] | 3 | 0 | 3 |
| P>F[y] | 5 | 3 | 8 |
| PI 126932 | 1 | 9 | 10 |
| Fla. 7776 | 10 | 0 | 10 |

[x] PI 126932 (scion) grafted to Fla. 7776(rootstock).
[y] Fla. 7776 (scion) grafted to PI 126932(rootstock).

# LIST OF REFERENCES


Ajith, A., T. Zhou, H.N. Trick, B.S. Gill, W.W. Bockus and S. Muthukrishnan, 2003. Greenhouse and field testing of transgenic wheat plants stably expressing genes for thaumatin-like protein, chitinase and glucanase against *Fusarium graminearum*. J. Exp. Bot., 54: 1101-1111.

Allard, R.W., 1960. Principles of plant breeding. 2nd Edn., New York: Wiley.

Alon, H., J. Katan and N. Kedar, 1974. Factors affecting penetrance of resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomatoes. Phytopathology, 64: 445-451.

Amadioha, A.C., 1993. A synergism between oxalic acid and polygalacturonases in the depolymerization of potato tuber tissue. World J. Microbiol. Biotechnol., 9: 599-600.

Aycock, R., 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*. N.C. Agric. Exp. Stn. Tech. Bull., pp: 174.

Bag, T.K., 2003. Two new orchid hosts of *Sclerotium rolfsii* Sacc. from India. New Disease Reports, 20: 8.

Barksdale, T.H. and A.K. Stoner, 1977. A study of the inheritance of tomato early blight. Plant Dis. Rep., 61: 63-65.

Barrett, J.T., 1934. Observations on the basidial stage of *Sclerotium rolfsii*. Phytopathology, 24: 1137-1138.

Bateman, D.F. and S.V. Beer, 1965. Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclerotium rolfsii*. Phytopathology, 55: 204-211.

Bateman, D.F., 1972. The polygalacturonase complex produced by *Sclerotium rolfsii*. Physiol. Plant Pathol., 2: 175-184.

Besler, B.A., A. Grichar and O.D. Smith, 1997. Reaction of selected peanut varieties and breeding lines to southern stem rot. Peanut Sci., 24: 6-9.

Besri, M., 2003. Tomato grafting as an alternative to methyl bromide in Marocco. Proceedings of the 2003 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, San Diego, CA, USA. Available from: http://mbao.org/2003/012%20besrimgraftingmbao2003sd.pdf [Accessed June 2010].

Boland, G.J. and T. Brimner, 2004. Nontarget effects of biological control agents. New Phytologist, 163: 455-457.

Boyle, L.W., 1952. Factors to be integrated in control of southern blight on peanuts. Phytopathology, 42: 282.

Bretó, M. P., M. Asins and A. Carbonell, 1993. Genetic variability in *Lycopersicon* species and their genetic relationships.Theor. Appl. Genet., 86: 113-120.

Brimner, T.A. and G.J. Boland, 2003. A review of the non-target effects of fungi used to biologically control plant diseases. Agricult. Ecosys. Environ., 100: 3-16.

Brown, J.E., C. Stevens, M.C. Osborn and H.M. Bryce, 1989. Black plastic mulch and spun bonded polyester row cover as method of southern blight control in bell pepper. Plant Disease, 73: 930-932.

Brunner, E., S. Domhof, and F. Langer, 2002. Nonparametric analysis of longitudinal data in factorial experiments. New York, John Wiley & Sons.

Budak, H., L. Cesurer, Y. Bolek, T. Dokuyuku and A. Akaya, 2002. Understanding of heterosis. J. Sci. Eng., 5(2): 68-75.

Cai, G., L.R. Gale, R.W, Schneider, H.C. Kistler, R.M. Davis, K.S. Elias and E. Miyao, 2003. Origin of race 3 of *Fusarium oxysporum* f.sp. lycopersici at a single site in California. Phytopathology, 93: 1014-1022.

California Department of Food and Agriculture (CDFA)., 1996. Methyl Bromide: An Impact Assessment. Office of Pesticide Consultation and Analysis, Sacramento, California.

Carlborg, O. and C.S. Haley, 2004. Epistasis: too often neglected in complex trait studies. Nat. Rev. Genet., 5: 618–625.

Cating, R., A. Palmateer and R. McMillan, 2009. Occurance of *Sclerotiumr rolfsii* on *Ascocentrum* and *Ascocenda* orchids in Florida. Phytopathology, 99(6): S19.

Chahal, G. and S. Gosal, 2002. Principles and procedures of plant breeding: biotechnological and conventional approaches. Alpha Science Int'l Ltd.

Chellemi, D., 1998. Alternative to methyl bromide in Florida tomatoes and peppers. The IPM Practitioner, 20(40): 1-6.

Cilliers, A.J., L. Herselman and Z.A. Pretorius, 2000. Genetic variability within and among mycelial compatibility groups of *Sclerotium rolfsii* in South Africa. Phytopathology, 90: 1026-1031.

Cook, R.J., W.L. Bruckart, J.R. Coulson, M.S. Goettel, R.A. Humber, R.D. Lumsden, J.V. Maddox, M.L. McManus, L. Moore, S.F. Meyer, P.C. Quimby, J.P. Stack and

J.L. Vaughn, 1996. Safety of microorganisms intended for pest and disease plant control: a framework for scientific evaluation. Biological Control, 7: 333-351.

Csinos, A. S., D.K. Bell, N.A. Minton and H.D. Wells, 1983. Evaluation of *Trichoderma* spp., fungicides, and chemical combinations for control of southern stem rot on peanuts. Peanut Science, 10: 75-79.

Curzi, M., 1931.Contributo alla conoscanza della biologia e della sistematica degli stipiti della. *Sclerotium rolfsii*. R. Accad. Lincei Randic., 15: 241-245.

Davey, A. E. and L.D. Leach, 1941. Experiments with fungicides for use against *Scelerotium rolfsii* in soils. Hilgerdia, 13: 523-547.

Deynze, A., K. Stoffel, R.R. Buell, A. Kozik, J. Liu, E. Knaap and D. Francis, 2007. Diversity in conserved genes in tomato. BMC Genomics, 8: 465.

Dutton, M.V. and C.S. Evans, 1996. Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. Can. J. Microbiol., 42: 881-895.

Duvick, N.D., 1996. Personal perspective plant breeding, an evolutionary concept. Crop Sci., 36(3): 539–548.

Edson, H.A. and N. Shapovalov, 1923. Parasitism of *Sclerotium rolfsii* on Irish Potatoes. Jour. Agr. Res., 23: 41-46.

Enivronmental Protection Agency. 2007. Available from: www.epa.gov/opprd001/rup/rup6mols.htm. [Accessed January 2010].

Epps, W.M., J.C. Patterson and I.E. Freeman, 1951. Physiology and paratism of *Sclerotium rolfsii*. Phytopathology, 41: 245-256.

Errakhi, R., P. Meimoun, A. Lehner, G. Vidal, J. Briand, F. Corbineau, J.P. Rona, F. Bouteau, 2008. Anion channel activity is necessary to induce ethylene synthesis and programmed cell death in response to oxalic acid. J. Exp. Bot., 59: 3121–3129.

Fajardo,T.G. and J.M. Mendoza, 1935. Studies on the *Sclerotium rolfsii* Sacc. attacking tomato, peanuts, and other plants in the Philippines. Philippine J. of Agr., 6: 387-424.

FAOSTAT-Agriculture, 2008. Available from: http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor. [Accessed March 2009].

Fery, R. and P. Dukes, 2005. Potential for utilization of pepper germplasm with a variable reaction to *Sclerotium rolfsii* Sacc. to develop southern blight-resistant pepper (*Capsicum annuum* L.) cultivars. Plant Genetic Resources, 3: 326–330.

Flores-Moctezuma, H.E., A. Montes-Belmont, R. Jimenez-Perez, R. Nava-Juarez, 2006. Pathogenic diversity of *Sclerotium rolfsii* isolates from Mexico, and potential control of southern blight through solarization and organic amendments, Crop Prot., 25: 195-201.

Freire, F.C.O., J.E. Cardoso, A. dos Santos, F.M.P. Viana, 2002. Diseases of cashew nut plants (*Anacardium occidentale* L.) in Brazil. Crop Prot., 21: 489–494.

Fulton, T.M., J. Chunwongse and S.D. Tanksley, 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol. Biol. Reptr., 13(3): 207-209.

Ganesan, S., R. Ganesh Kuppusamy and R. Sekar, 2007. Integrated management of stem rot disease (*Sclerotium rolfsii*) of groundnut (*Arachis hypogaea* L.) using rhizobium *and Tricoderma harzianum* (ITCC-4572). Turk. J. Agric. For., 31: 103-108.

Garren, K.H., 1959. The stem rot of peanuts and its control. Virginia Agr. Exp. Sta. Bull., 144.

Glazebrook, J., 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 2005. 43:205–27.

Gordon, A. and A. Taylor, 1941. The Photolysis of Methyl Bromide. J. Am. Chem. Soc., 63(12): 3435-3441.

Goto, K., 1935. *Sclerotium rolfsii* Sacc. in perfect stage III. Variation in cultures originated from basidiospores. Jour. Soc. Trop. Agr. Formosa, 7: 331-345.

Hassan, A.A. and K.E.A. Abdel-Ati, 1999. Genetics of Tomato yellow leaf curl virus tolerant derived from *Lycopersicon pimpinellifolium* and *Lycopersicon pennellii*. Egypt. J. Hortic., 26: 323-338.

Hassan, A.A., H.M. Mazayd, S.E. Moustafa, S.H. Nassar, M.K. Nakhla and W.L. Sims, 1984. Genetics and heritability of tomato yellow leaf curl virus tolerance derived from *Lycopersicon pimpinellifolium*. European Association for Research on Plant Breeding. Tomato Working Group. Wageningen, Netherlands, pp: 81-87.

Higgins, B.B., 1923. The disease of pepper. Georgia Agr. Exp. Sta. Bull., 141: 48-75.

Higgins, B.B., 1927. Physiology and parasitism of *Sclerotium rolfsii* Sacc. Phytopathology, 17: 417-448.

Higgins, B.B., 1934. Important disease of pepper in Georgia. Georgia Agr. Exp. Sta. Bull., 186: 1-20.

Howard, P. H., 1991. Handbook of environmental fate and exposure data for organic chemicals: Pesticides. CRC Press.

Hudgins, H.R., 1952. Relation of nitrogen concentration to the development of southern blight of peanuts. M.S. Thesis, Texas Agr. and Mechanical Coll., U.S.A.

Hutton, S.F., 2008. 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. Ph.D. Thesis, University of Florida, U.S.A.

Inami, S. and S. Suzuki, 1981. Breeding alfalfa, *Medicago sativa* L., for southern blight resistance. I. Varietal differences of the disease injury. J. Jpn. Soc. Grassland Sci. 26: 360-364.

Inami, S., M. Kanbe and F. Fujimoto, 1986. Breeding varieties of lucerne with resistance to southern blight. III. Increase in resistance according to advance of selection generation and heritability values. J. Jpn. Soc. Grassland Sci., 32: 218-224.

Jeeva, M.L., V. Hegde, T. Makeshkumar, R.R. Nair and S. Edison, 2005. *Dioscorea alata*, a new host of *Sclerotium rolfsii* in India. New Disease Reports, 10: 49.

Jenkins, S.F. and C.W. Averre, 1986. Problems and progress in integrated control of southern blight of vegetables. Plant disease, 70(7): 614-619.

Jha, G. and K. Thakur, 2009. The Venturia apple pathosystem: Pathogenicity mechanisms and plant defense responses. J. Biomed. Biotechnol., 2009: 1-10.

Ji, Y., D.J. Schuster, and J.W. Scott, 2007. Ty-3, a begomovirus resistance locus near the tomato yellow leaf curl virus resistance locus Ty-1 on chromosome 6 of tomato. Mol. Breeding, 20: 271-284.

Jones, J.B., J.P. Jones, R.E. Stall, and T.A. Zitter, 1991. Compendium of tomato diseases. Amer. Phytopathol. Soc., St. Paul, Minnesota.

Kasrawi, M. A. and A. Mansour, 1994. Genetics of resistance to tomato yellow leaf curl virus in tomato. J. Hortic. Sci., 69: 1095-1100.

Kim, K., J.Y. Min and M.B. Dickman, 2008. Oxalic Acid Is an Elicitor of Plant Programmed Cell Death during *Sclerotinia sclerotiorum* Disease Development. Mol. Plant-Microbe Interact., 21: 605-612.

Latunde-Dada, A.O., 1993. Biological control of southern blight disease of tomato caused by *Sclerotium rolfsii* with simplified mycelial formulations of *Trichoderman koningii*. Plant Pathology, 42: 522-529.

Leach, L.D. and A.E. Davey, 1942. Reducing southern Scelrotium rot of sugar beets with nitrogenous fertilizers. J. Agr. Res., 64: 1-18.

Lee, J.M. and M. Oda, 2003. Grafting of herbaceous vegetables and ornamental crops. Hort Rev., 28: 61-124.

Lee, J.M., 2003. Advances in vegetable grafting. Chronica Hort., 43: 13-19.

Leeper, P., S. Phatak, D. Bell, B. George, E. Cox, G. Oerther and B. Scully, 1992. Southern blight resistant tomato breeding lines: 5635M, 5707M, 5719M, 5737M, 5876M, and 5913M. HortScience, 27(5): 475-478.

Lehner, A., P. Meimoun, R. Errakhi, K. Madiona, M. Barakate and F. Bouteau, 2008. Toxic and signaling effects of oxalic acid. Plant Signaling & Behavior, 3: 746-748.

Linderman, G.R. and G.R. Gilbert, 1973. Behavior of sclerotia of *Sclerotium rolfsii* produced in soil or in culture regarding germination stimulation by volatiles, fungistasis, and sodium hypochlorite treatment. Phytopathology, 63: 500-503.

Liua, B., D. Glennb and K. Buckleyc, 2008. Trichoderma communities in soils from organic, sustainable, and conventional farms, and their relation with Southern blight of tomato. Soil Biol. Biochem., 40: 1124–1136.

Livingstone, D., J.L. Hampton, P.M. Phipps and E.A. Grabau, 2005. Enhancing resistance to Sclerotinia minor in peanut by expressing a barley oxalate oxidase gene. Plant Physiol., 137: 1354-1362.

Lyle, J.A.,1953. A comparative study of *Sclerotium rolfsii* Sacc. and *Sclerotium delphinii* Welch. Ph.D thesis (unpublished). University of Minnesota, U.S.A.

McColloch, L.P., H.T. Cook and W.R. Wright, 1968. Market disease of tomatoes, peppers, and eggplants. Agr. Hdbk. no.28, U.S. Dept. Agr. Res. Serv., Washington, D.C., U.S.A.

Milthorpe, F.L., 1941. Studies on *Corticium rolfsii* (Sacc.) Curzi (*Sclerotium rolfsii* Sacc.) I. Cultural characters and perfect stage. II. Mechanism of parasitism. Proc. Linn. Soc., 66: 65-75.

Mohr, H.C. and G.M. Watkins, 1959. The nature of resistance to southern blight in tomato and the influence of nutrition on its expression. Proc. Amer. Soc. Hort. Sci., 74: 484-493.

Mohr, H.C., 1955. Resistance in *Lycopersicon pimpinellifolium* Mill. to southern blight caused by *Sclerotium rolfsii* Sacc. Ph.D. Thesis, M699, Texas A&M University, U.S.A.

Mohr, H.C., V.A. Greulach and A.A. Dunlap, 1947. Recent studies of southern blight and root know of tomatoes. Tex. Agr. Exp. Sta. Prog. Rept., 1092.

Muller, C.H., 1940. A revision of the genus Lycopersicon. U.S.Dept. Agr. Misc. Publ. No, 382, pp: 1-29.

Mundkur, B.B., 1934. Perfect stage of *Sclerotium rolfsii* Sacc. in culture. Indian Jour. Agr. Sci., 4: 779-781.

Nalim, F.A., J.L. Starr, K.E. Woodard, S. Segner and N.P. Keller, 1995. Mycelial compatibility groups in Texas peanut field populations of *Sclerotium rolfsii*. Phytopathology, 85: 1507-1512.

Naqvi, S.A.M.H., 2004. Disease of fruits and vegetables: Diagnosis and Management. Volume1. Kluwer Academic Publishers.

Nash, A.F. and R.G. Gardner, 1988a. Heritability of tomato early blight resistance derived from *Lycopersicon hirsutum* PI 126445. J. Am. Soc. Hort. Sci., 113: 264-268.

Nisikado, Y., K. Hirata and T. Higuti, 1938. Studies on the temperature relations to the longevity of pure culture of various fungi, pathogenic to plants. Ber. Ohara. Inst., fur. Landwirtschaftliche. Forschungen, 8(2): 107-124.

Parvez, S., 2006. Recent advances in understanding genetic basis of heterosis in rice (*Oriza sativa* L.). Revista Científica UDO Agrícola, 6: 1-10.

Pérez de Castro, A., M. J. Díez and F. Nuez, 2007. Inheritance of Tomato yellow leaf curl virus resistance derived from *Solanum pimpinellifolium* UPV16991. Plant Dis., 91: 879-885.

Phatak, S.C. and K.D. Bell, 1983. Screening for *Sclerotium rolfsii* resistance in the tomato.In: Proc.4th Tomato Quality Wrkshp, Veg. Crops Res.Rpt., VEC-83-1, Dept. Veg. Crops, Inst. Food and Agr. Sci., Univ. of Florida, Gainesville. pp: 107.

Pimental, D., and A. Wilson, 2004. World population, agriculture, and malnutrition. World Watch Magazine, 17(5): 22-25.

Polizzi, G., D. Aiello, V. Guarnaccia, G. Parlavecchio and A. Vitale, 2010. First report of southern blight on silverbush (*Convolvulus cneorum*) caused by *Sclerotium rolfsii* in Italy. Plant Dis., 94: 131.

Povah, A., 1927. Notes on reviving old cultures. Mycologia, 19: 317-319.

Pratt, R. G. and D.E. Rowe, 2002. Enhanced resistance to *Sclerotium rolfsii* in populations of alfalfa selected for resistance to *Sclerotinia trifoliorum*. Phytopathology, 92: 204-209.

Punja, Z.K. and L.J. Sun, 1997. Genetic diversity among mycelial compatibility groups of *Sclerotium rolfsii* and *Sclerotium delphini*. In: Programme and summaries of the 11th biennial conference of the Australian Plant Pathology Society. Perth, Australia, 29th September-2nd October 1997, pp: 110.

Punja, Z.K. and R.G. Grogan, 1982. Effects of inorganic salts, carbonate-bicarbonate anions, ammonia, and the modifying influence of pH on sclerotial germination of *Sclerotium rolfsii*. Phytopathology, 72: 635-639.

Punja, Z.K., 1985. The biology, ecology and control of *Sclerotium rolfsii*. Ann. Rev. Phytopathol. 23: 97-127.

Punja, Z.K., S.F. Jenkins and R.G. Grogan, 1984. Effect of volatile compounds, nutrients, and source of sclerotia on eruptive sclerotial germination of *Sclerotium rolfsii*. Phytopathology, 74: 1290-1295.

Ragsdale, N. N. and W.B. Wheeler, 1995. Methyl bromide risks, benefits, and current status in pest control. In: Review of Pesticide Toxicology, R. M. Roe and R. J. Kuhr (Eds.). Raleigh, North Carolina: Toxic Communication Inc., pp: 21-44.

Retig, N., N. Kedar and J. Katan, 1967. Penetrance of gene I for Fusarium resistance in the tomato. Euphytica, 16: 252-257.

Reyes-Valde´s, M.H., 2000. A model for marker-based selection in gene introgression breeding programs. Crop Sci., 40: 91-98.

Ribaut, J.M. and D. Hoisington, 1998. Marker-assisted selection: New tools and strategies. Trends Plant Sci., 3: 236-239.

Rick , C.M., 1958. The role of natural hybrization in the derivation of cultivated tomatoes in western South America. Econ. Bot., 12: 346-367.

Rick, C. M., 1988. Molecular markers as aids for germplasm management and use in *Lycopersicon*. HortScience., 23 : 55-57.

Ristaino, J.B., K.B. Perry and R.D. Lumsden, 1991. Effect of solarization and *Gliocladium virens* on sclerotia of *Sclerotium rolfsii*, soil microbiota, and incidence of southern blight on tomato. Phytopathology, 81: 1117-1124.

Rivard, C.L. and F.J.Louws, 2008. Grafting to manage soil-borne disease in heirloom tomato production. HortScience, 43(7): 2104-2111.

Rivard, C.L., F.J. Louws, S. O'Connell and M.M. Peet, 2009. Grafting tomato with inter-specific rootstock provides effective management for southern blight and root-knot nematodes. Phytopathology, 99(6): S109.

Rodriguez-Kabana, R., K.M. Beute and A.P. Backman, 1980. A method for estimating numbers of viable sclerotia of *Sclerotium rolfsii* in soil. Phytopathology, 70(9): 917-919.

Rosen, H.R., 1929. Studies *on Sclerotium rolfsii* with special reference to the metabolic interchange between soil inhabitants. Arkansas. Agr. Exp. Sta. Annu. Rep., pp: 66.

Saccardo, P.A., 1911. Notes mycologicae. Ann. Mycel., 9: 252-261.

Salvador R., M.J. Díez and F. Nuez, 1998. Genetics of tomato spotted wilt virus resistance coming from *Lycopersicon peruvianum*. Eur. J. of Plant Pathol., 104: 499-509.

Sankaran, K., E.M. Florence and J. Sharma, 2007. Two new diseases of forest tree seedlings caused by *Sclerotium rolfsii* in India. Eur. J. For. Pathol., 14(4-5): 318-320.

Sconyers, L.E., T.B. Brenneman, K.L. Stevenson and B.G. Mullinix, 2005. Effects of plant spacing, inoculation date, and peanut cultivar on epidemics of peanut stem rot and tomato spotted wilt. Plant Dis., 89(9): 969-974.

Semagn, K., A. Bjornstad and M. N. Ndjiondjop, 2006. Progress and prospects of marker assisted backcrossing as a tool in crop breeding programs. Afri. J. Biotechnol., 5 (25): 2588-2603.

Shah, D.A. and L.V. Madden, 2004. Nonparametric analysis of ordinal data in designed factorial experiments. Phytopathology 94:33-43.

Sharma, B. K., U.P. Singh, K.P. Singh, 2002. Variability in Indian isolates of *Sclerotium rolfsii.* Mycologia, 94(6): 1051-1058.

Sharma, D. and N.S. Jodha, 1984. Pulse production in Semi-arid region of India. Proceedings of Pulses Production, Constraints and Opportunities. pp. 241-265.

Sherf, A.F. and A.A. MacNab, 1986. Vegetable diseases and there controls. John Wiley & Sons.

Shukla, R. and A. K. Pandey. 2008. Pathogenic diversity of *Sclerotium rolfsii* isolates, a potential biocontrol agent against *Parthenium hysterophorus L*. Afr. J. Environ. Sci. Technol., 2: 124-126.

Shukla, R. and A.K. Pandey, 2007. Diversity in mycoherbicidal agent *Sclerotium rolfsii* isolates from Central India. J.Mycol. Pl. Pathol., 37(3): 514-518.

Sleper, D.A. and J.M. Poehlman, 2006. Breeding field crops. 5th Edn., Blackwell Publishing.

Smith, A.M., 1972. Drying and wetting sclerotia promotes biological control of *Sclerotium rolfsii* Sacc. Soil. Biol. Bio-chem., 4: 125-129.

Sofi, P.A., A.G. Rather and K. Warsi, 2007. Implications of epistasis in maize breeding. Int. J. Plant Breed. Genet., 1: 1-11.

Soller, M., T. Brody and A. Genizi, 1976. On the power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. Theor. Appl. Genet., 47: 35-39.

Soumpourou, E., M. Lakovidis, L. Chartrain, V. Lyall and C. Thomas, 2007. The *Solanum pimpinellifolium* Cf-ECP1 and Cf-ECP4 genes for resistance to *Cladosporium fulvum* are located in the Milky Way locus on the short arm of chromosome 1. Theor. Appl. Genet., 115: 1127-1136.

Spreen, T. H., J.J. Van Sickle, A.E. Moseley, M.S. Deepak, and L. Mathers, 1995. Use of methyl bromide and the economic impact of its proposed ban on the Florida fresh market fruit and vegetable industry. Bull. Univ. Fla. Exp. Stn. No. 898.

Sugha, S.K., B.K. Sharma and P.D. Tyagi, 1991. A modified technique for screening chickpea (*Cicer arietinum*) varieties against collar rot caused by *Sclerotium rolfsii*. Indian J. Agric. Sci., 61(4): 289-290.

Suliman-Pollatschek, S., K. Kashkush, H. Shats, and U. Lavi, 2002. Generation and mapping of AFLP, SSRs and SNPs in *Lycopersicon esculentum*. Cell. Mol. Biol. Lett., 7: 583-597.

Tanksley, S. and S. McCouch, 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science, 277(5329): 1063–1066.

Tanksley, S.D., N.D. Young, A.H. Paterson and M.W. Bonierbale, 1989. RFLP mapping in plant breeding: new tools for an old science. BioTechnology, 7: 257-264.

Taubenhaus, J.J., 1919.Recent studies on *Sclerotium rolfsii* Sacc. J. Agr. Res., 18(3): 127-138.

Thangavelu, R. and M.M. Mustaffa, 2010. First report of corm rots disease caused by *Sclerotium rolfsii* in banana. Australasian Plant Disease Notes, 5(1): 30-33.

Tigchelaar, C.E., 1986. Tomato breeding. In: Breeding for Vegetable Crops, Bassett, M.J. (Eds.)., AVI, Westport, Conn., pp: 135-171.

United Nations Environment Programme (UNEP), 1995. 1994 Report of the methyl bromide technical options committee. Montreal Protocol on substances that deplete the ozone Layer. United Nations Ozone Secretariat, Nairobi, Kenya.

United States Department of Agriculture (USDA), 2008. Available from: www.nass.usda.gov/Statistics_by_Subject/index.asp [Accessed May 2009].

United States Department of Agriculture,1993. The Biological and Economic Assessment of Methyl Bromide. Available from: http://pmep.cce.cornell.edu/profiles/fumigant/methyl_bromide/methbrom_rsk_0193.html. [Accessed May 2009].

University of Georgia. 2005. Available from http://pubs.caes.uga.edu/caespubs/pubcd/SB41-07/SB41-07.html#Pecan [Accessed May 2009].

Vallad, G.E., Q.M. Qin, R. Grube, R.J. Hayes and K.V. Subbarao, 2006. Characterization of race-specific interactions among isolates of *Verticillium dahlia* pathogenic on lettuce. Phytopathology, 96: 1380-1387.

VanSickle, J.J. and A. Hodges, 2008. U.S. production trends and the impact of the Florida fresh market tomato industry to the economy of Florida. Food and resource economics department, Florida cooperative extension service, IFAS, University of Florida. Published September 2008. Available from http: //edis.ifas.ufl.edu. [Accessed March 2009].

Vos, P., R. Hogers and M. Bleeker, 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research, 23(21): 4407–4414.

Wang S., C.J. Basten and Z.B. Zeng, 2010. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. Available from http://statgen.ncsu.edu/qtlcart/WQTLCart.htm [Accessed January 2010].

Watkins, G.M., 1950. Germination of sclerotia of *Sclerotium rolfsii* after storage at various relative humidity levels. Phytopathology, 40: 31.

Weber,G.F. and G.B. Ramsay, 1926.Tomato disease in Florida. Florida Agr. Exp. Sta. Bull., 185: 61-138.

West, E., 1961. *Sclerotium rolfsii*, history, taxonomy, host range and distribution. Phytopathology, 51: 108-109.

Yang, W., S.A. Miller, J.W. Scott, J.B. Jones and D.M. Francis, 2005. Mining tomato genome sequence databases for molecular markers application to bacterial resistance and marker assisted selection. Acta Hort., 695: 241-250.

Yang, W., X. Bai, E, Kabelka, C. Eaton, S. Kamoun, E.D.F. Van der Knaap, 2004. Discovery of single nucleotide polymorphisms in *Lycopersicon esculentum* by computer aided analysis of expressed sequence tags. Molecular Breeding, 14: 21-24.

Young, N.D. and S.D. Tanksley, 1989a. RFLP analysis of the size of chromosomal segments retained around the tm-2 locus of tomato during backcross breeding. Theor. Appl. Genet., 77: 353-359.

Young, N.D., D. Zamir, M.W. Ganal and S.D. Tanksley, 1988. Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the *Tm-2a* gene in tomato. Genetics, 120: 579-585.

Young, P. A., 1946. Tomato disease in Texas. Texas Agr. Exp. Sta. Circ., pp: 113.

Young, P.A., 1954. Experimental control of southern blight on tomato. Plant Dis. Reptr., 38: 858.

# BIOGRAPHICAL SKETCH

Mehul Samir Bhakta was born in Navsari, India. Influenced by his grandfather and brother he decided to pursue a B.S. degree in agricultural science. He graduated from Navsari Agricultural University earlier known as Gujarat Agricultural University in the year 2006. Being fascinated by the advancement in genetics and the changes it could bring to agriculture he joined Dr. Jeremy Edwards's tomato genetics and plant breeding program at University of Florida in the year 2008 as a master's student.