GREENHOUSE EVALUATION OF SOYBEAN FOR RESISTANCE TO SCLEROTINIA STEM ROT AND QUANTITATIVE TRAIT LOCI STUDY IN RECOMBINANT INBRED LINES

By

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ABSTRACT

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Sclerotinia stem rot [caused by *Sclerotinia sclerotiorum* (Lib) de Bary] is an economically important disease of soybean [*Glycine max* (L.) Merr] and no soybean cultivars show complete resistance to the disease. To screen soybean cultivars and lines for resistance to this disease, three related but independent studies were conducted in the greenhouse and laboratory. In the first study, 392 $F_{4:6}$ recombinant inbred lines (RILs) from seven populations were evaluated for resistance to *S. sclerotiorum* by drop- and spray-mycelium methods under the greenhouse conditions. Individual lines in two of seven populations evaluated by drop-mycelium method were significantly different ($P<0.0500$). Parental polymorphism was tested with 132 simple sequence repeat (SSR) markers associated with Sclerotinia stem rot resistance in other studies and 97 polymorphic markers were used to test the progenies from the seven populations. Sixteen markers showed high correlations with the phenotypic data in the seven populations. In the second study, 66 plant introductions (PIs) were evaluated with the drop-mycelium method and significant ($P < 0.0050$) differences were found among the PIs for resistance to Sclerotinia stem rot. In the third study, drop-mycelium, spray-mycelium, and field evaluation methods were compared in terms of correlation of the data. The data from drop-mycelium inoculation had strong correlations with that from spray-mycelium ($R^2 = 0.63, P< 0.0005$) and field evaluations ($R^2 = 0.40, P<0.0381$) for resistance to Sclerotinia stem rot.

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CHAPTER ONE

STUTY OF QUANTITATIVE TRAIT LOCI IN SOYBEAN FOR RESISTANCE TO SCLEROTINIA STEM ROT

ABSTRACT

Sclerotinia stem rot [caused by *Sclerotinia sclerotiorum* (Lib) de Bary] is considered an economically important disease of soybean [*Glycine max* (L.) Merr]. Some soybean cultivars show partial resistance to Sclerotinia stem rot but no complete resistance to Sclerotinia stem rot has been reported. The objectives of this study were to evaluate seven populations for resistance to Sclerotinia stem rot under the greenhouse condition and validate the quantitative trait loci (QTLs) associated with Sclerotinia stem rot resistance in soybean. Seven populations with a total of 392 recombinant inbred lines (RILs) of soybean were developed by crossing Skylla, a partial resistant cultivar, and E00290, a susceptible cultivar with five plant introductions (PIs): PI 089001, PI 153259, PI 437764, PI 548404, and PI 548312 that exhibit partial resistance to Sclerotinia stem rot. The 392 $F_{4:6}$ RILs from the seven populations were evaluated for resistance to *S. sclerotiorum* by drop and spray-mycelium methods in the greenhouse conditions. Individual lines in populations one and seven were significantly different ($P<0.0235$ and $P<0.0019$, respectively) in levels of resistance obtained with the drop-mycelium method. Parental polymorphism was tested with 132 simple sequence repeat (SSR) markers associated with Sclerotinia stem rot resistance found in previous studies and 97 polymorphic markers were used to screen the progenies from the seven populations. Sixteen markers were identified to highly correlate with phenotypic data in the seven populations. Markers such as Sat_267, Satt651, Satt571, Satt619, and Satt475 showed significant correlations in more than one population. Satt494, Satt154, Satt197, Satt481, Satt394, Satt197, Satt243, Satt153, Satt478, and Satt691 markers were significant in individual populations.

INTRODUCTION

Soybean, [*Glycine max* (L.) Merr.] is the second most important crop in terms of area and production in the United States (US). It belongs to the genus, *Glycine*, which is divided into two subgenera; *Glycine* and *Soja*. The subgenus *Soja,* include the cultivated soybean, *G. max,* and the wild progenitor of *G. max, G. soja. G.max* and *G. soja* are cross-compatible.

Sclerotinia stem rot, caused by necrotrophic homothallic fungal pathogen, *Sclerotinia sclerotiorum* (Lib.) De Bary, is a major soybean disease in the north-central areas of the United States (Hartman et al., 1998). Sclerotinia stem rot was first found in the US in 1946 and reported in 1951 but outbreaks of the disease became more frequent and more severe after 1990s (Yang et al., 1999). *S. sclerotiorum* is capable of colonizing over 400 species of plants including soybean (Boland and Hall, 1994). The pathogen requires wet soil and canopy conditions at flowering for infections to occur (Grau, 1988). Sclerotia are the primary long-term survival structures and play a major role in disease cycle (Willets and Wong, 1980). Sclerotia germinate carpogenically or myceliogenically depending on environmental conditions. Myceliogenic germination of sclerotia produces mycelia that can directly attack plant tissue (Le Tourneau, 1979) while carpogenic germination produces apothecia and subsequently ascospores (Bardin and Huang, 2001). Airborne ascospores are the primary inoculums for disease development and senescent flowers are the primary infection sites (Cline and Jacobsen, 1983; Abawi and Grogan, 1979). Infection of soybean plants occurs during the reproductive phase of soybean plant growth. Ascospores that land on flower petals germinate when free water is present on plant surfaces, utilizing the petal as a nutrient base (Kurle et al., 2001). Infection starts with colonization of petals and mycelium

spreads to pods, nodes, and stems and may result in premature plant death (Grau and Radke, 1984). The typical foliar symptoms of Sclerotinia stem rot include necrotic leaves, lesions on stem and pods, white fluffy mycelia, and black sclerotia present on the plant surface and internally in the stems and pods (Chen and Wang, 2005).

Sclerotinia stem rot caused estimated yield loss of 235 kg/ha (Chun et al., 1987) and 147 to 370 kg ha^{-1} (2-5 bu $acre^{-1}$) for every 10% increase in disease severity, depending on the environment and cultivar (Grau et al., 1982). Hoffman et al. (1998) reported that Sclerotinia stem rot of soybean caused a significant reduction in seed size, seed oil content, seed germination, and seed quality. Sclerotia are often harvested inadvertently along with the seed and can cause reduced seed quality as well as broader distribution of the pathogen (Grau et al., 2004; Danielson et al., 2004). Sclerotinia stem rot can cause as much yield loss as soybean cyst nematode (*Heterodera glycines* Ichinohe) and Phytophthora root and stem rot (*Phytophthora sojae* Kauffman and Gerdemann) when environmental conditions are conducive (Grau et al., 2004; Arahana et al., 2001). Sclerotinia stem rot ranks fifth after Soybean Cyst Nematode, Phytophthora root rot, seedling diseases, and brown stem rot (Wrather and Koernning, 2006).

Sclerotinia stem rot in soybean is difficult to control due to pathogen's wide host range in combination with its persistent resting structures, sclerotia (Phillips, 1989). Solarization reduced the populations of *S. sclerotiorum* and ability of the surviving sclerotia significantly at 10 and 15 cm depths (Philips, 1990). The prevalence of Sclerotinia stem rot was less in no-till than in minimum-till or conventional-till fields. In addition, the prevalence was greater in minimum-till than in conventional-till fields (Workneh and Yang, 2000). Cultural practices like use of narrow row spacing, higher plant density, and optimal fertilizer application create a dense plant canopy,

which in turn favors high humidity leading to fungal infection and disease outbreak (Mueller et al., 2004). Crop management practices such as use of clean seeds, early planting date, soil tillage, and adjustment of row width and plant density contribute to a reduction in Sclerotinia stem rot severity, but the effectiveness of these measures can be very limited (Steadman, 1979; Muller et al., 2002). These management practices recommended for controlling the Sclerotinia stem rot in soybean were found to be ineffective and contrary to the high yield potential of soybean (Kim and Diers, 2000). The widespread occurrence of Sclerotinia stem rot is due to changes in management practices, planting susceptible germplasm, and weather conditions that favor disease development (Kurle et al., 2001).

Dann et al. (1999) found significant reductions in disease severity after treatment of soybean plants with lactofen at the R1 growth stage, and yields were higher after treatment with 0.07 and 0.11 kg a.i. ha^{-1} lactofen compared with water control. Foliar-applied fungicide benomyl aids in the control of Sclerotinia stem rot in dry beans when applied at 10 percent bloom, but this practice has not been thoroughly tested in soybeans (Scott et al., 1998). Benomyl, thiophanate methyl, and vinclozolin applied to soybean seedlings at V2 growth stage in greenhouse condition prevented *S. sclerotiorum* from expressing symptoms or signs on leaf tissue. Vinclozolin was the most effective in inhibiting *S. sclerotiorum* mycelia growth at 1.0 µg a.i. ml of potato dextrose agar (Mueller et al., 2004). The disease pressure must be sufficient to justify the application of fungicides indicating a little value in applying fungicides when fewer than 25 percent of the plants become infected (Venete, 1998). The effectiveness of fungicides to control Sclerotinia stem rot in soybean has been shown to be inconsistent (Grau et al., 1994; Mueller et al., 2002), due to difficulties in achieving good coverage with fungicides and timing of application with regard to ascospore release (Hunter et al., 1978; Steadman, 1979). Chemical

control is not economically viable for controlling Sclerotinia stem rot of soybean due to the requirement of many preventative and systemic treatments (Mueller et al., 2004).

Oxalic acid is the main pathogenic factor of *S. sclerotiorum* (Cessna et al., 2000). Soybean plants inserted with transgene that produces oxalate oxidase (oxalic acid degrading enzyme) showed disease severity index (DSI) as low as resistant commercial cultivars, and in addition, showed very low DSI as compared to non-transgenic line in fields infested with *S. sclerotiorum* (Cober et al., 2003). Soybean plants transformed with a wheat germin gene (gf-2.8) greatly reduced the Sclerotinia stem rot, providing evidence that wheat germin gene (gf-2.8) degrades oxalic acid produced by *S. sclerotiorum* (Donaldson et al., 2001). Livingstone et al. (2005) transformed peanut plants with a barley oxalate oxidase gene. Transgenic peanut plants reduced the lesion size by 75% to 97% compared to non-transgenic plants, providing evidence that oxalate oxidase can confer resistance to Sclerotinia blight in peanut. Hu et al. (2003) found that sunflower plants transformed with a wheat OXO gene exhibited enhanced resistance against *S. sclerotiorum*. Dias et al. (2006) transformed lettuce (*Lactuca sativa*) with decarboxylase gene (oxdc) isolated from a *Flammulina sp*. The transgenic lettuce plants either had no symptoms or had slow disease development in comparison with a non-transgenic control line for resistance to *S. sclerotiorum*. However, transgenes have the potential risk of escaping into the environments (Burke and Rieseberg, 2003).

Host resistance is the most economical and long-term strategy for controlling the Sclerotinia stem rot in soybean (Grau et al., 1982). But Current sources of resistance to Sclerotinia stem rot show only partial resistance, and are limited in number within soybean germplasm (Hoffman et al., 1998). Other researchers also have reported that soybean accessions

and cultivars do not show complete resistance to Sclerotinia stem rot (Hartman et al., 2000; Hoffman et al., 2002; Kim et al., 1999) but show partial resistance in the field, greenhouse (Nelson et al., 1991), and growth room evaluations (Boland and Hall, 1986). Use of partial resistance varieties is the most effective way to enhance the yield of soybean (Kim and Diers, 2000). Partial resistance to *S. sclerotiorum* is inherited as a quantitative trait in soybean (Kim and Diers, 2000; Vuong et al., 2008) and common bean (Miklas et al., 2004). Hoffman et al. (1999) suggested that inheritance of partial resistance is controlled by single recessive allele. Kim and Diers (2000) suggested a multi-locus model to define the genetics of soybean cultivars for showing differential susceptibility to Sclerotinia stem rot. Mestries et al. (1998) found that resistance to *S. sclerotiorum* in sunflower was polygenic and complex. Arahana et al. (2001) argues that genetic complexity of the trait and the variability in disease development in field evaluations make it difficult for breeding resistance to Sclerotinia stem rot. Partial resistance to *S. sclerotiorum* is composed of physiological resistance and disease escape mechanism in the field evaluations (Kim and Diers, 2000; Rousseau et al., 2004). Planting cultivars that are physiologically resistant to Sclerotinia stem rot is the most effective way to manage the disease due to difficulties in controlling the environmental conditions (Kurle et al., 2001).

Molecular markers are powerful tools for breeders to find new sources of resistant QTLs or alleles (Song et al., 2004). Rongwen et al. (1995) argues that morphological and pigmentation markers have limited potential to distinguish the uniqueness of new soybean cultivars. SSR markers, composed of tandemly repeated 2-5 base pair DNA sequences, have flanking DNA sequences that are generally conserved allowing the selection of polymerase chain reaction (PCR) primers which amplify the SSR markers. Akkaya et al. (1992) reported that SSR markers are abundant and highly polymorphic in soybean. One soybean SSR locus has as many as 23

alleles, which provides the evidence of high level of polymorphism shown by SSR markers that helps in dissecting genetics of soybean (Cregan et al., 1994) and defining linkage group homology across mapping populations unambiguously (Cregan et al., 1999).

Miklas et al. (2000) states that breeding for genetic resistance is complex since it is conditioned by both physiological and avoidance mechanisms. Thus developing varieties with partial resistance to *S. sclerotiorum* is a major goal of soybean breeding programs. Quantitative trait loci (QTLs) are the parts of DNA that are closely linked to the genes that underlie a quantitative trait. Quantitative trait loci analysis is a statistical method that attempts to explain genetic basis of complex traits (Lynch and Walsh, 1998). There have been many studies to identify the QTLs associated with resistance to Sclerotinia stem rot in soybean germplasm. Low lignin concentration in the stem of soybean is positively correlated with lower disease severity and suggested that stem lignin concentration can be used as a biological marker for selection of soybean lines for resistance to Sclerotinia stem rot (Peltier et al., 2009). Open plant architecture, early maturity, and upright architecture of the soybean cultivars caused inconsistent disease ratings in the field (Kim et al., 2000). But reactions of soybean to Sclerotinia stem rot in the greenhouse or laboratory evaluations are due to physiological resistance with little chance of escape mechanisms (Grau and Bissionette, 1974; Nelson et al., 1991).

Arahana et al. (2001) identified twenty-eight putative QTLs that confer partial resistance to Sclerotinia stem rot in five RIL populations encompassing 15 linkage groups but the amount of phenotypic variation explained by each QTL was less than 10%. Kim and Diers (1999) discovered three QTLs in 152 F_3- derived soybean lines developed from a cross between a partially resistant cultivar, NKS19-90, and a susceptible cultivar, Williams 82, associated with

resistance to Sclerotinia stem rot with each QTL explaining less than 10% of the total phenotypic variation. Li et al. (2010) reported three QTLs on two linkage groups associated with partial resistance to Sclerotinia stem rot, each QTL explaining less than 16% of the total phenotypic variation. Guo et al. (2008) reported seven QTLs associated with resistance to *S. sclerotiorum* in two PIs 391589A and 391589B. Vuong et al. (2008) identified four QTLs on four linkage groups (LGs A2, B2, K, and L) associated with resistance to Sclerotinia stem rot, each QTL explaining less than 13% phenotypic variation. Quantitative trait loci mapped on LG A2 is located 12 cM from a QTL reported by Han et al. (2007) in the patent application. Huynh et al. (2010) identified three QTLs on two different linkage groups (LGs C2 and I) of soybean associated with resistance to Sclerotinia stem rot. Guo et al. (2008) argues that favorable alleles of QTLs identified in different studies that are associated with resistance to Sclerotinia stem rot can be used for resistance gene pyramiding. Diers et al. (2006) derived Soybean cultivar AxN-1-55 from a cross of two partially resistant cultivars Asgrow A2506 and NKS-1990. AxN-1-55 had lower disease ratings than A2506 or S19-90. Wang et al. (2006) developed a cultivar Skylla, partially resistant to Sclerotinia stem rot from the cross Dairylan 'DSR-217' x NKS19-90. Skylla was developed by advancing F1 plants to F4 using single-seed descent. It had disease severity index (DSI) ratings lower than resistant check cultivar NKS19-90 and higher than Dwight, a susceptible cultivar.

Soybean plant introductions (PIs) are mostly used as sources of pest resistance in back-crossing breeding programs, but not as sources of genes for yield improvement programs. Over half of the genetic base of North American soybeans is derived from less than fifty plant

introductions (Delannay et. al., 1983). Shands and Wiesner (1991) pointed out that germplasm in major crops have been primarily used to identify single gene sources of resistance to diseases and insects or tolerance to abiotic stresses. In addition, germplasm have been introgressed to increase the genetic base and variability in adapted cultivars. A study has shown a linear increase in yield as the percentage of germplasm from PIs decrease in intermated populations, but greatest amount of genetic variability for yield was observed when the intermated populations had fifty percent PI germplasm (Schoener and Fehr, 1979). PIs of soybean may enhance crop genetics for yield improvement (Thorne and Fehr, 1970; Vello et al., 1984). Soybean germplasm collection may be a rich source of alternative alleles (Li et al., 2008). About 6,520 soybean PIs from maturity group 0 to IV were evaluated for resistance to Sclerotinia stem rot in the US and Canada both in the field and greenhouse conditions. Only sixty-eight PIs were selected as partially resistant PIs based on their reactions to *S. sclerotiorum* (Hoffman et al., 2002).

Reactions of soybean cultivars to Sclerotinia stem rot in the field conditions are confounded by escape mechanisms posing difficulties in identifying physiological resistance (Boland and Hall, 1987) whereas reactions in the controlled conditions are largely due to physiological resistance (Nelson et al., 1991). Since the environment has a large role in the development of Sclerotinia stem rot in soybean, it is very important to control the environment when attempting to map QTL associated with physiological resistance (Kim and Diers, 2000; Vuong et al., 2008). Different inoculation methods have been developed to screen soybean cultivars for resistance to Sclerotinia stem rot in a greenhouse or laboratory including; cotyledon inoculation (Grau and Bissonette, 1974; Kull et al., 2003), excised stem or detached leaf assay (Chun et al., 1987, Steadman et al., 2001; Wegulo et al., 1997), cut-stem inoculation (Kull et al., 2003; Vuong et al., 2003), cut-petiole inoculation (del Rio et al., 2001), and drop- and spray-

mycelium method (Chen and Wang, 2005). The reaction of soybean cultivars to Sclerotinia stem rot showed significant correlation between greenhouse and field data (Kim et al., 2000). Chen and Wang (2005) suggested that drop and spray-mycelium are non-destructive, low cost, and efficient methods for evaluation of soybean germplasm and breeding lines for resistance to Sclerotinia stem rot in greenhouse or controlled conditions.

Skylla, a partially resistant cultivar (Wang et al., 2006) and E00290, a susceptible cultivar, were crossed with 5 partially resistant Plant Introductions (PI 089001, PI 153259, PI 437764, PI 548404, and PI 548312) from Hoffman et al. (2002) to derive seven populations (Table 1) with a total of 392 recombinant inbred lines (RILs) and they were evaluated in the greenhouse for resistance to *S. sclerotiorum.* The locations of QTLs in these five PIs are crucial for future soybean breeding programs. If we could locate the position of QTLs in these sources, that knowledge can be used for pyramiding resistance genes in developing soybean cultivars with high level of resistance to Sclerotinia stem rot. If the QTLs in these resistance sources are not co-localized with any reported QTLs, it should carry new resistant QTLs. Our objectives were to a) evaluate a total of 392 RILs in greenhouse for resistance to Sclerotinia stem rot and b) validate the already reported QTLs associated with resistance to Sclerotinia stem rot in these PIs.

MATERIALS AND METHODS

PHENOTYPIC ANALYSIS

DROP-MYCELIUM METHOD

Seven soybeans $F_{4:6}$ RIL populations were evaluated in the greenhouse conditions by drop-mycelium method as described by Chen and Wang (2005) for resistance to *S. sclerotiorum.*

A total of 392 lines were planted with a resistant check (NKS19-90) and a susceptible check (Olympus) in different dates (Table 2). Six seeds were planted in each 10cm x 10cm x 15cm plastic pot. The pots were arranged in a randomized complete block design with 3 replications in each population. Clear plastic 32-ounce PET cups with the bottoms removed were put upside down over each pot to keep the plant upright. Plants were allowed to germinate and reach to V-3 growth stage before inoculation was carried out. When the plant mortality of susceptible (Olympus) check was about 100%, data collection was performed.

INOCULUM PREPARATION

Fungal inoculums were prepared from the sclerotia obtained from the previous year. The sclerotia were surface-sterilized with 10% bleach. Sterilized sclerotia were grown in potato dextrose agar (PDA) medium for 3-4 days. The mycelia on the PDA plates were cut in small pieces and transferred into liquid potato dextrose broth medium. To facilitate quick and even mycelial growth, the liquid medium was shaken by a G10 GYROTORY shaker for 96 hours. The mycelium suspension was homogenized by blending in a household blender. The mycelium suspension (approximately 1 ml) was applied at the unfolded trifoliate leaves at V3 growth stage. The misting chamber was equipped with humidifiers, which constantly provided almost 100% humidity required for disease development. Seven to ten days after inoculation when the susceptible checks had a mortality of over 80%, the total number of dead plants per pot for each line was counted and plant mortality rate was calculated as follows;

Plant mortality (PM) = number of dead plants/ total number of plants in pot

The PROC GLM procedure of SAS (SAS, 2008) was used to calculate the significant difference between lines within the population. The broad-sense heritability for significantly

different populations was calculated with the variance component method described by Fehr (1987). The variance components were estimated with PROC GLM of SAS (SAS Institute, Cary, NC) using the statistical model: $Y_{ij} = \mu + G_i + R_j + GR_{ij} + \varepsilon_{ij}$ where Y_{ij} is the observed phenotypic value of ith genotype (i = 1...., 59, and 324….., 392) in j^{th} replication (j = 1, 2, 3), μ is the overall mean, G_i is the effect of genotype, R_j is the effect of replication, GR_{ij} is the interaction of genotype by replication, ε_{ij} is the plant-to-plant variation within the replication.

SPRAY-MYCELIUM METHOD

For spray-mycelium method, the 392 lines and the resistant (NKS19-90) and susceptible (Olympus) checks were planted in 2 replications (Table 1.3). Each line had two pots in two replications. Six seeds per line were planted in 10cm x 10cm x 15 cm plastic pots filled with Baccto porous potting mix. Planting, spraying, and data collection dates are found in Table 1.2. Plants were inoculated at V3 growth stage.

In order to keep plant upright in the pots, 32 ounce clear plastic PET cups with the bottoms removed were placed upside down over all pots. The pots were arranged in randomized complete block design. Two semi-opaque plastic chambers housed the two benches containing pots. The chambers remained open until inoculation. Each chamber had two humidifiers at the end of bench. Humidifiers were set to a 2-minute on, 3 minute off regime 24 hours a day.

Inoculum was prepared with the same methodology as in drop-mycelium method. But inoculum suspension was applied by a battery operated hand sprayer. Plant mortality data were collected on day 14 after inoculation.

TISSUE SAMPLING, DNA EXTRACTION AND SSR MARKER GENOTYPING

Tender leaves from 392 lines were collected and stored at -80 degree Celsius for two days before lypholization. The lyophilized tissue was ground by vigorous shaking with glass beads in 15-ml tubes with a paint shaker. The DNA was extracted with the CTAB (hexadecyltrimethyl ammonium bromide) method as described by Kisha et al. (1997) and the DNA concentration was measured with a ND-1000 Spectrophotometer (NanoDrop Technologies, Inc, Wilmington, Delware). The PCR was performed in MJ TetradTM thermal cycler (MJ Research, Waltham, MA). the PCR products were separated on 6% non-denaturing polyacrylamide gels using an electrophoresis unit DASG-400-50 (C.B.S. Scientific Co. DelMar, CA) as described by Wang et al. (2003). Ethidium bromide was used to stain the gel and PCR products were visualized under UV light, and photographed. A total of 132 simple sequence repeat (SSR) primer pairs (SOYBASE) were selected for the parental polymorphism flanking already reported 33 QTLs from the integrated soybean linkage map (Song et al., 2004; Choi et al., 2007). Genotyping with SSR markers was carried out as described by Wang et al. (2003). These SSR markers (Table 9) were tested for polymorphism between seven parental combinations and about 97 polymorphic markers were scored on the seven populations. For each polymorphic marker, the DNA bands of each RIL were scored as ‘a’, ‘b’ or ‘h’, where ‘a’ means only band of the resistant parent present, ‘b’ means only band of the susceptible parent present, and ‘h’ means band of the both parents present. Polymerase Chain Reactions (PCR) were performed for DNA amplification.

The populations as shown in the Table 1.1 were genotyped with polymorphic SSR markers from regions containing 32 reported QTLs. The selected SSR were tested with the

parent DNA of each population for polymorphism according to Wang et al. (2003). The markers which show polymorphism between the two parents were then used to genotype the entire populations (Appendix, Table 3). The phenotypic data obtained from the greenhouse experiment were analyzed with the genotypic data obtained from marker analysis to determine if the DNA markers are associated with resistance to the disease in these seven populations. Single marker analysis was carried out to determine the marker-resistance association.

RESULTS AND DISCUSSION

DROP-MYCELIUM METHOD

Among the seven populations studied, soybean lines in population one and population seven showed significant difference ($P< 0.0235$ and $P<0.0019$ respectively) among one another (Table 1.6). Soybean lines in the other populations were not significantly different. The mortality rate of soybean lines in seven populations varied from 0 to 100 percent. NKS 19-90 and Olympus were used as resistant and susceptible checks, respectively. Average plant mortality for seven populations ranged from 18.7 % to 57.4 %. The variation in plant mortality among the populations is expected due to differences in genetic contributions by different parents, different planting dates, and varying ambient temperature.

SPRAY-MYCELIUM METHOD

For the spray-mycelium method, the effect of evaluation date, individual population, and individual line were accounted. Evaluation date and population were significant ($P<0.001$). Individual lines had no significance ($P<0.2633$). The significance in evaluation date signifies that

there was marked difference in the percentage of survival between evaluation dates. Since each evaluation date had different populations, this difference was as expected. A significant difference in survival between populations is also expected due to different genetic backgrounds of their parents. Plant mortality distributions of all populations are displayed in the appendix. Average plant mortality for individual lines ranged from 29.1% in population 7 to 79.8% in population 3. Variation in mortality distribution is show in Fig. 1.2. Populations 4, 5, and 6 were evaluated twice, once with cup modification 25 February evaluation, represented by bars in charts) and once without the cup modification (17 December evaluation, represented by the line in charts) shown in Fig. 1.3. Plants fell over after sprayed allowing a more aggressive spread of Sclerotinia stem rot since infections occurred in multiple places on the plant in without cup modification evaluation. Data collection was performed at ten days after inoculation because disease developed rapidly and caused plants to die early. Since true resistance response elicited by the plant is better measured if the disease progressed downward from infection point, we used cup to keep the plant upright. The rest of the evaluations were carried out by cup modifications. Evaluation with the cup modification was done fourteen days after inoculation as described by Chen and Wang (2005). Further analysis (Table 1.7) was performed to look at the variance between replications of a single population in only one evaluation date. Under this analysis, populations 2 and 4 in the Dec, 2007 evaluation were significant for survival variation ($P<.0189$, $P<.0212$ respectively). Since the lines were derived from same parents, the variation is explained by the effects of the genotype. These populations were also grouped by the Duncan method to determine the division of significant differences between lines (Table 1.8). Since all of the lines of a population were not planted during the same evaluation date. This explains why only twenty lines in population 2 and eight lines in population 4 considered in the Duncan groupings. Seven

of the twenty lines in population 2 were not significantly different and only two groups had individuals that were independently significant from all other groups. Population 4 had one line that was significantly different than the remaining seven lines. It is interesting to note that the without cup evaluation is the only one giving significant difference between lines. This further provides evidence that allowing the infection to grow for 14 days is too long to get good results. The spray-mycelium method is effective to identify the significant differences between lines but it has some demerits too. During the spraying process, pieces of mycelia often clogged the spray nozzle, increasing the time to apply the inoculums. Because there was high error rate with this method, additional studies should be conducted taking the data multiple times throughout the disease growth period to determine the best growth period.

Number of plants with higher plant mortality is more for spray-mycelium method (Fig. 1.2). Plant mortality is normally or near normally distributed for populations 2, 4, 5, and 6. Variation in plant mortality distribution for drop-mycelium method is less than spray-mycelium method. This also indicates that drop-mycelium method is more uniform and more reliable.

Kim and Diers (2000) estimated the broad-sense heritability of Sclerotinia stem rot resistance at 0.59 in a 152 F_3- derived lines from S19-90 crossed with Williams 82. Miklas and Grafton (1992) estimated the broad-sense heritability in three populations of common bean for resistance to Sclerotinia stem rot that ranged from 0.58 to 0.77. The broad-sense heritability in our populations 1 and 7 were 0.59 and 0.60, respectively (Table 1.5). Grau et al. (1982) concluded that field resistance to soybean Sclerotinia stem rot is a heritable trait. Previous studies of broad-sense heritability of Sclerotinia stem rot resistance trait in soybean along with our study suggest that partial resistance to Sclerotinia stem rot in soybean is heritable trait.

GENOTYPIC RESULT

Altogether 97 SSR markers (Table 1. 10) were polymorphic for seven populations covering 15 linkage groups of soybean consensus map (Song et al., 2004). A total of 5 markers were polymorphic across all seven populations. Some markers are polymorphic in two or more populations. Since there were few markers per population per linkage group, increasing the marker density in $\pm$ 20 cM region of the polymorphic markers would help better construct the linkage map and detect the QTLs associated with it. The correlation coefficients between population and polymorphic markers identified in this study are depicted in the Table 1.8. It is difficult to conclude whether seven populations used in this study possess the already reported QTLs, but this study gave us some insights on which region of chromosome our future study should concentrate.

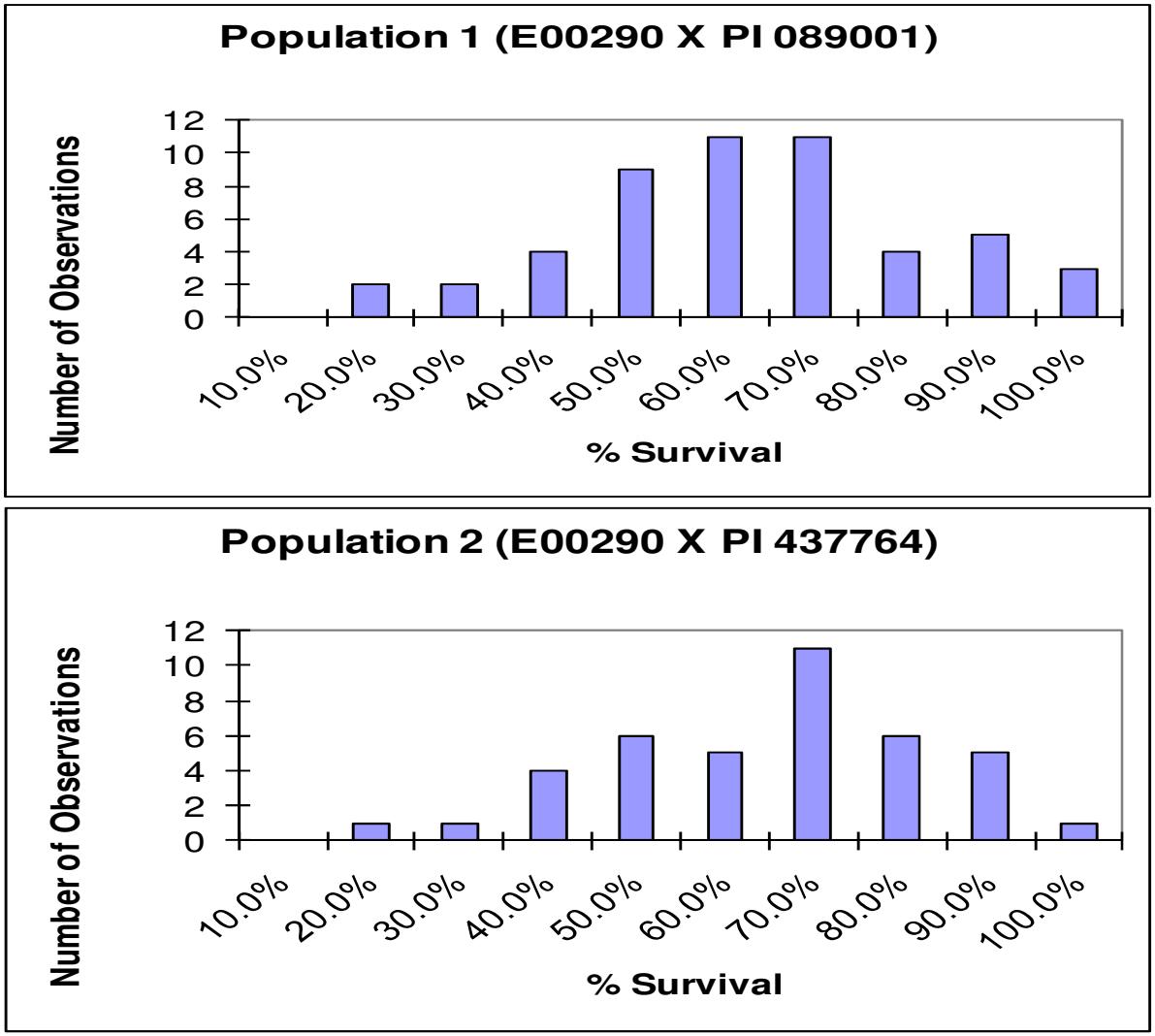


FIGURE 1.1- SURVIVAL DISTRIBUTIONS OF THE 7 POPULATIONS OBTAINED FROM SPRAY-MYCELIUM. POPULATIONS 4, 5, AND 6 REPRESENT BOTH NON-CUP AND CUP MODIFICATION RESULTS. (FOR INTERPRETATION OF THE REFERENCES TO COLOR IN THIS AND ALL OTHER FIGURES, THE READER IS REFERRED TO THE ELECTRONIC VERSION OF THIS THESIS.)

FIGURE 1.1(CONT'D)

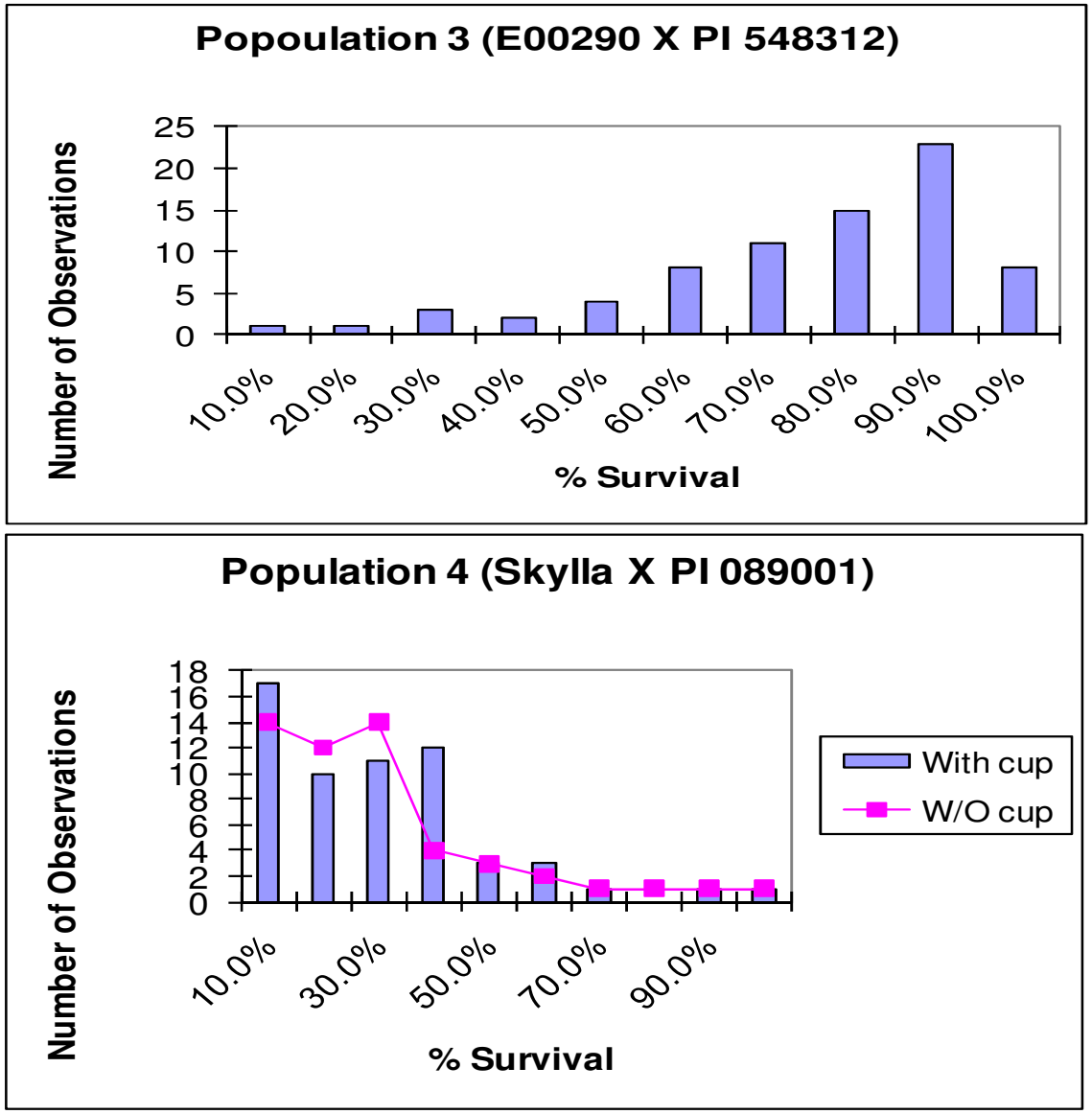
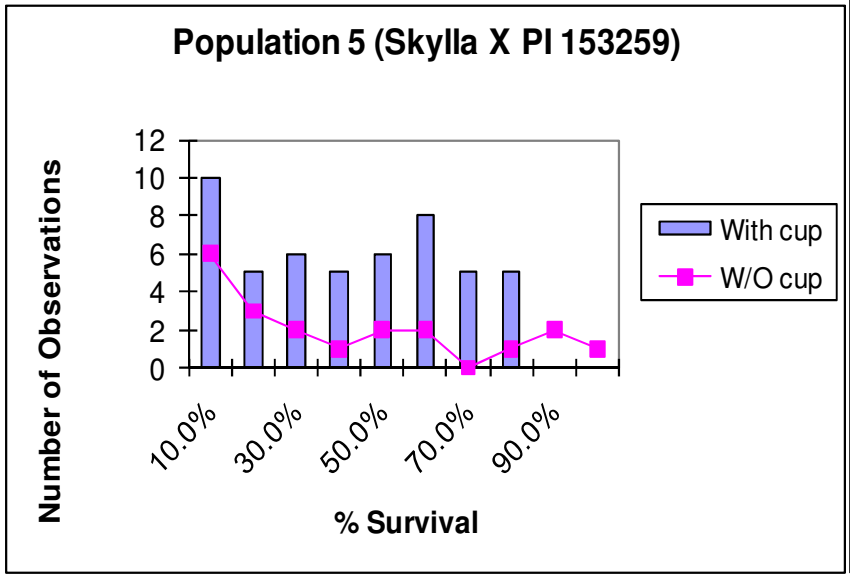
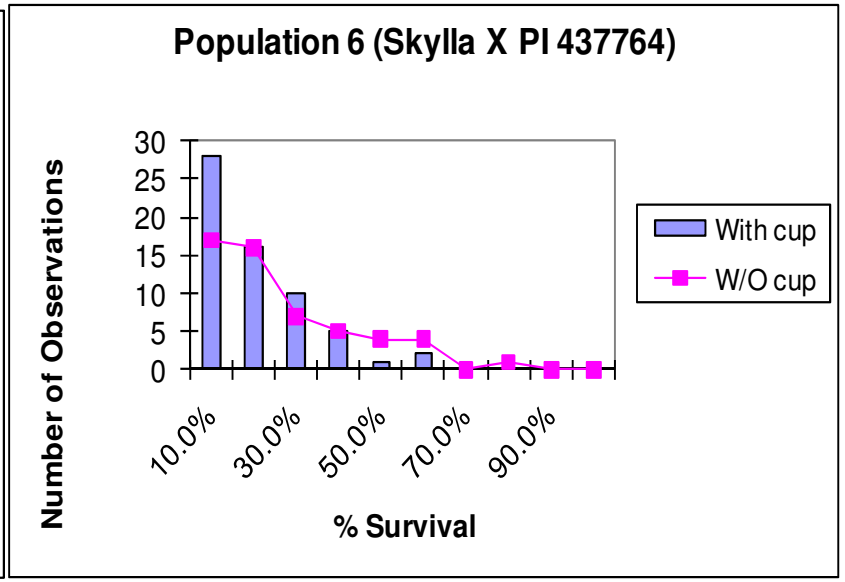
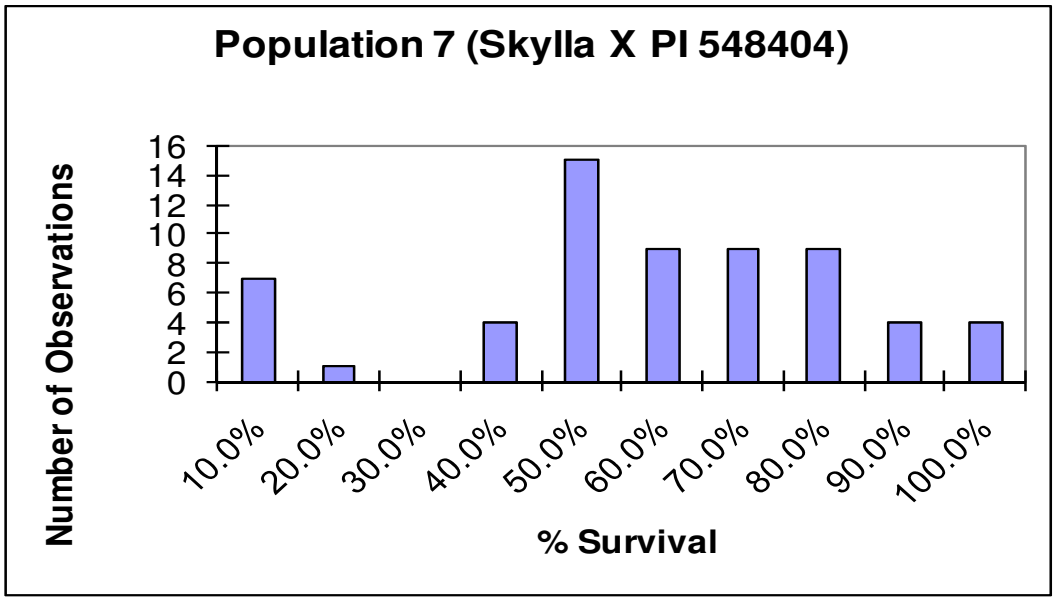


FIGURE 1.1 (CONT'D)







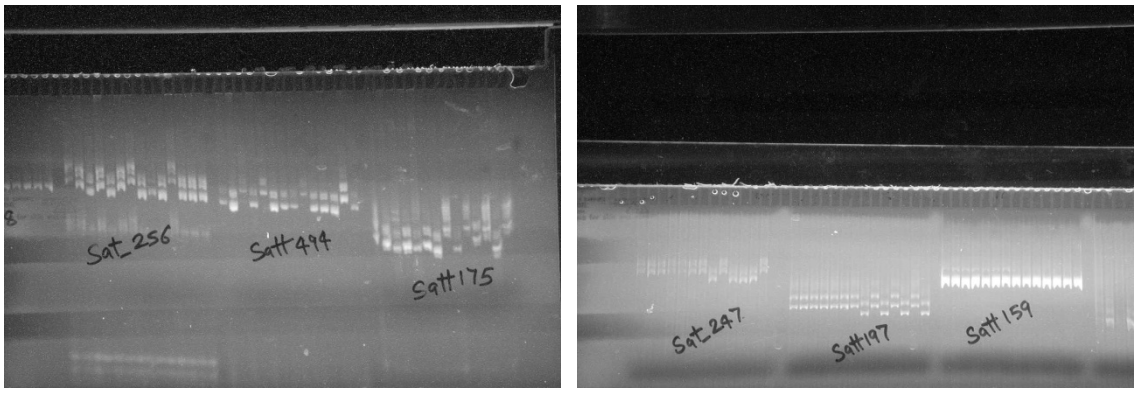


FIGURE 1.2- SSR MARKERS POLYMORPHIC AND NON-POLYMORPHIC IN DIFFERENT PARENTS

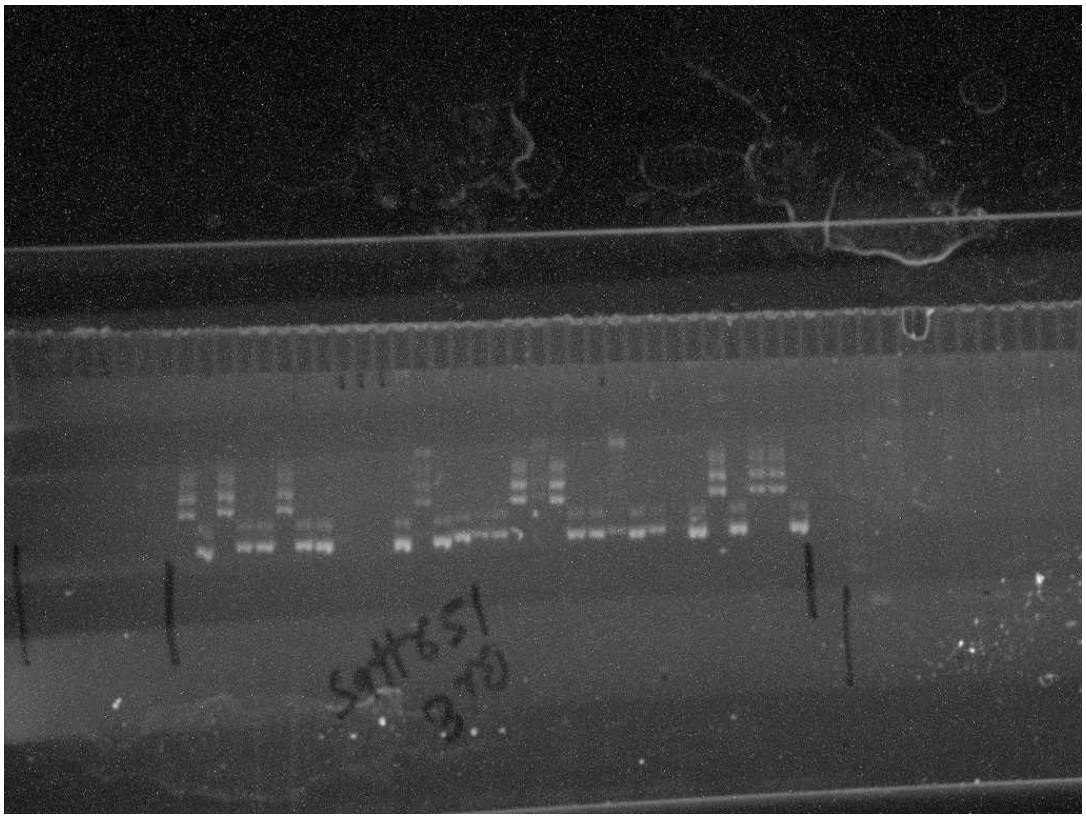


FIGURE 1.3- MARKERS SHOWING POLYMORPHISMS IN POPULATION

TABLE 1.1- LISTING OF CROSSES USED IN THIS STUDY AND NUMBER OF LINES IN EACH CROSS

Population	female parent	male parent	Number of lines
1	E00290[1]	PI89001	59
2	E00290	PI437764	50
3	E00290	PI548312[3]	63
4	Skylla[2]	PI89001[3]	62
5	Skylla	PI153259[3]	51
6	Skylla	PI437764[3]	38
7	Skylla	PI548404[3]	69

[1] Susceptible to *Sclerotinia* stem rot

[2] carries resistance to Sclerotinia stem rot from NKS19-90

[3] partially resistant to Sclerotinia stem rot obtained from Hoffman et al., (2002)

TABLE 1.2- LIST OF CROSSES AND MAJOR EVENTS CARRIED OUT IN THE GREENHOUSE (DROP-MYCELIUM METHOD)

Population	Crosses	Planting	Inoculation	Data taken
1	a) Skylla × PI 153259 b) Skylla × PI437764 c) E00290 × PI 437764	Dec 12, 2008	Jan 4, 2009	Jan 14, 2009
2	a)Skylla × PI 089001 b)PI 548404 × E00290	Dec 27, 2008	Jan 23, 2009	No data taken
3	a)E00290 × PI548312 b)E00290 × PI089001	Jan 11, 2009	Feb 7, 2009	Feb 15, 2009
4	a) Skylla × PI08900124 b) Skylla × PI 548404	Oct 16, 2009	Nov 12, 2009	Nov 20 and Nov 21

TABLE 1.3- COMPLETE LIST OF MAJOR EVENTS OCCURRING DURING SCREENING OF THE 7 POPULATIONS (SPRAY-MYCELIUM METHOD)

Plant date	Spray date	Data Collection
9 Nov, 2007	6 Dec, 2007	17 Dec, 2007
27 Nov, 2007	24 Dec, 2007	7 Jan, 2008
14 Dec, 2007	14 Jan, 2007	28 Jan, 2008
12 Jan, 2008	11 Feb, 2008	25 Feb, 2008

TABLE 1.4- GLM PROCEDURE OF SAS OUTPUT FOR SEVEN POPULATIONS EVALUATED BY DROP-MYCELIUM METHOD

Populations	source	DF	Type III SS	Mean Square	F- Value	Pr > F
1	Pid	58	62726.36306	1058.95270	1.49	0.0345
2	Pid	50	39118.35659	782.36713	0.87	0.7038
3	pid	63	55724.46742	884.51536	0.95	0.5878
4	pid	61	64193.02086	1052.34460	1.13	0.2764
5	pid	50	42167.21149	843.34423	0.97	0.5311
6	pid	37	47313.59417	1278.74579	1.38	0.1202
7	pid	68	103734.8964	1525.5132	1.51	0.0216

TABLE 1.5- RESULTS OF VARIANCE ANALYSIS AND BROAD-SENSE HERITABILITY ESTIMATES FOR RESISTANCE TO SCLEROTINIA STEM ROT

Source of variation	Mean Square	
	Population 1	**population 7**
Genotype	1081.4890	1525.5132
Error	724.0448	1011.2664
Heritability	0.59	0.60

TABLE 1.6- SIGNIFICANCE DIFFERENCE BETWEEN LINES IN POPULATION 1AND 7 SHOWN BY LEAST SIGNIFICANT DIFFERENCE (LSD)

	pop 7			**pop 1**	
t-grouping at α=0.05	**mean**	**Pid**	**t-grouping at α=0.05**	**mean**	**pid**
A	95.2	359	A	84.1	8
Ab	93.3	361	Ab	60.0	7
Abc	68.8	343	Ab	55.6	1
Abcd	67.7	352	Ab	55.6	6
Bcdefg	42.1	324	Abc	48.9	45
Cdegfh	41.6	376	Bc	33.3	59
Defghi	16.6	364	Bc	33.3	29
Fghij	5.5	337	C	0.0	53
			C	0.0	42

TABLE 1.7- SIGNIFICANCE DIFFERENCE BETWEEN LINES WITHIN 7 POPULATIONS BY SPRAY-MYCELIUM METHOD

	Dec 17, 2007	**Jan 7, 2007**	**Jan 28, 2007**	**Feb 25, 2007**
Population	*P*	*P*	*P*	*P*
1	.5520		.2266	.7795
2	.0189		.7952	.5027
3	.6797		.7055	.4526
4	.0212	.6091	.2110	.5425
5		.8124	.0685	
6		.1942	.3560	
7		.3946	.3435	.6280

TABLE 1.8- SIGNIFICANT DIFFERENCE WITHIN LINES OF 17 DEC, 2007 EVALUATION

Population 4			Population 2		
ID[2]	**Mean**	**Duncan group**	**ID**	**Mean**	**Duncan group**
184	0.6365	A	91	0.4745	a
178	0.1805	B	82	0.4575	ab
186	0.1750	B	93	0.4320	abc
183	0.0910	B	88	0.2865	abcd
179	0.0000	B	102	0.2535	abcd
185	0.0000	B	89	0.2265	abcd
180	0.0000	B	61	0.2080	abcd
187	0.0000	B	94	0.1820	abcd
			77	0.1780	abcd
			76	0.1705	abcd
			62	0.1540	cbd
			83	0.1415	cbd
			87	0.1130	Cd
			60	0.0555	D
			85	0.0415	D
			73	0.0415	D
			75	0.0000	D
			67	0.0000	D
			65	0.0000	D
			64	0.0000	D

[2] Plant ID

TABLE 1.9- REPORTED QTLS ASSOCIATED WITH PARTIAL RESISTANCE TO SCLEROTINIA STEM ROT IN SOYBEAN

SSR locus	LG	cM Position in LG	GenBank Accession	GenBank GI Number	Repeat motif
Satt619	A1	69.21	CC453983	31044813	(ATT)11
Satt545*	A1	71.39	BH126713	14970216	(ATT)19
Sat_267	A1	78.45	CC453802	31044632	(AT)32
Satt424*	A2	60.59	BH126603	14970106	(ATT)52
Satt212	E	32.27	BH126418	14969921	(ATT)10
Satt341	A2	77.7	BH126532	14970035	(ATT)17
Satt197*	B1	46.39	BH126404	14969907	(ATT)20
Satt638	B1	37.8	CC453997	31044827	(ATT)13
Sat_247	B1	49.73	CC453785	31044615	(AT)21
Satt070*	B2	72.81	BH126318	14969821	(ATT)24
Sat_189	B2	72.92	CC453730	31044560	(AT)10
Satt122	B2	72.46	BH126336	14969839	(ATT)8
Satt147*	D1a	108.89	BH126359	14969862	(ATT)14
Satt129	D1a	109.67	BH126343	14969846	(ATT)26

TABLE 1.9(CONT'D)

SSR Locus	LG	cM Position in LG	GenBank Accession	GenBank GI Number	Repeat motif
Satt459*	D1b	118.62	BH126632	14970135	(ATT)13
Satt274	D1b	116.35	BH126470	14969973	(ATT)18
Sat_202	D1b	118.86	CC453743	31044573	(AT)17
Satt256*	D2	124.31	BH126454	14969957	(ATT)10
Sat_022	D2	120.3	BH126254	14969757	(AT)27
Satt386	D2	125	BH126571	14970074	(ATT)15
Satt720*	E	20.8	CC454064	31044894	(ATT)19
Satt651	E	32.1	CC454006	31044836	(ATT)10
Satt691	E	19.7	CC454043	31044873	(ATT)17
Sat_317*	F	72.97	CC453848	31044678	(AT)24
Satt510	F	71.41	BH126681	14970184	(ATT)21
Sat_120	F	75.97	BH126290	14969793	(AT)31
Satt191*	G	96.57	BH126398	14969901	(ATT)18
Sat_117	G	100	BH126287	14969790	(CT)6(CA)8'(AT)9
Satt472	G	94.84	BH126644	14970147	(ATT)37

* reported QTLs

TABLE 1.9 (CONT'D)

SSR locus	LG	cM Position in LG	GenBank Accession	GenBank GI Number	Repeat motif
Satt451*	I	20.34	BH126625	14970128	(ATT)10
Satt419	I	21.9	BH126598	14970101	(ATT)22
Satt571	I	18.5	BH126737	14970240	(ATT)14
Satt588*	K	117.02	BH126754	14970257	(ATT)18(AT)10(CT)14
Sat_126	K	108.2	BH126296	14969799	(AT)17
Satt481*	L	54.57	BH126653	14970156	(ATT)14
Sat_340	L	55.51	CC453866	31044696	(AT)31
Sat_150	L	53.67	CC453702	31044532	(AT)24
Satt494*	M	71.71	BH126665	14970168	(ATT)13
Sct_147	M	73.88	BH126779	14970282	(CT)10
Satt175	M	66.99	BH126384	14969887	(ATT)16
Satt387*	N	53.25	BH126572	14970075	(ATT)10
Satt549	N	70.6	BH126716	14970219	(ATT)29
Sat_266	N	47.28	CC453801	31044631	(AT)30
Sat_109*	O	127.5	CC453690	31044520	(AT)28
Sat_231	O	128.44	CC453770	31044600	(AT)22

TABLE 1.9 (CONT'D)

SSR locus	LG	cM Position in LG	GenBank Accession	GenBank GI Number	Repeat motif
Sat_307	O	123.43	CC453839	31044669	(AT)34
Sat_233*	A2	86.42	CC453772	31044602	(AT)14
Satt301*	D2	93.71	BH126492	31044710	(ATT)24
Satt458*	D2	24.52	BH126631	14970134	(ATT)31
Satt154*	D2	57.07	BH126366	14969869	(ATT)20
Sat_092	D2	57.51	CC453687	31044517	(AT)31
Satt582	D2	53.85	BH126748	14970251	(ATT)16
Satt114*	F	63.69	BH126332	14969835	(ATT)17
Sat_234	F	66.55	CC453773	31044603	(AT)22
Sat_229	F	62.79	CC453768	31044598	(AT)21
Satt394*	G	43.38	BH126577	14970080	(ATT)31
Satt115	G	43.78	BH126333	14969836	(ATT)18
Satt273*	K	56.62	BH126469	14969972	(ATT)13
Satt725	K	56.85	CC454067	31044897	(ATT/ATT)25
Sat_111	K	55.7	BH126281	14969784	(AT)16
Satt260*	K	80.12	BH126458	14969961	(ATT)22
Sat_167	K	85.19	CC453714	31044544	(AT)23

TABLE 1.9 (CONT'D)

SSR locus	LG	cM Position in LG	GenBank Accession	GenBank GI Number	Repeat motif
Satt475	K	78.68	BH126647	14970150	(ATT)16
Sat_134*	L	28.27	BH126304	14969807	(AT)35
Sat_405	L	29.62	CC453929	31044759	(AT)33
Satt523	L	27.92	BH126693	14970196	(ATT)15
Satt009*	N	28.52	BH146212	15243078	(ATT)14
Satt478*	O	71.1	BH126650	14970153	(ATT)17
Sat_242	O	74.05	CC453780	31044610	(AT)18
Satt563	O	68.39	BH126729	14970232	(ATT)18
Satt243*	O	119.5	BH126444	14969947	(ATT)17
Sat_307	O	123.43	CC453839	31044669	(AT)34
Sat_109	O	127.5	CC453690	31044520	(AT)28
Satt172*	D1b	100.89	BH126381	14969884	(ATT)9

TABLE 1.10- LIST OF POLYMORPHIC SSR MARKERS ACROSS SEVEN POPULATIONS AND THEIR RESPECTIVE LINKAGE GROUPS

	SSR markers	Linkage Groups	cM position
Across all populations	Sat_267	A1	78.45
	Satt619	A1	69.21
	Satt651	E	32.1
	Satt571	I	18.5
	Sat_244	M	48.86
E00290 × PI89001	Satt153	O	118.4
	Satt243	O	119.5
	Sat_109	O	127.5
	Satt478	O	71.1
	Sat_242	O	74.05
	Satt494	M	71.71
	Sat_256	M	74.53
	Sat_092	D2	57.51
	Sat_229	F	62.79
	Satt154	D2	57.07
	Sat_234	F	66.55
	Satt475	K	78.68
	Satt641	N	29.28
	Sat_340	L	55.51
	Satt481	L	54.57
	Sat_199	A2	84.09
	Satt186	D2	105.45
E00290× PI437764	Sat_199	A2	84.09
	Satt186	D2	105.45

TABLE 1.10 (CONT'D)

	SSR markers	Linkage Groups	cM position
	Sat_340	L	55.51
	Satt641	N	29.28
	Sat_092	D2	57.51
	Satt494	M	71.71
	Sat_256	M	74.53
	Sat_236	N	57.59
	Sat_109	O	127.5
	Satt153	O	118.14
	Satt243	O	119.5
	Sat_022	D2	120.3
	Satt691	E	19.7
E00290×Pi548312	Satt451	I	20.34
	Satt243	O	119.5
	Sat_109	O	127.5
	Sat_236	N	57.59
	Sat_234	F	66.55
	Satt260	K	80.12
	Satt641	N	29.28
	Satt159	N	27.13
	Sat_340	L	55.51
	Satt481	L	54.57
	Satt523	L	27.92
	Satt394	G	43.38
	Satt147	D1a	108.89
Skylla × PI89001	Satt197	B1	46.39
	Sat_247	B1	39.73

TABLE 1.10 (CONT'D)

	SSR markers	Linkage Groups	cM position
	Sat_340	L	55.51
	Satt154	D2	57.07
	Sat_234	F	66.55
	Sat_092	D2	57.51
	Satt494	M	71.71
	Sat_236	N	57.59
	Satt243	O	119.5
	Satt153	O	118.4
	Satt691	E	19.7
Skylla × PI 153259	Satt691	E	19.7
	Satt472	G	94.84
	Satt153	O	118.4
	Satt478	O	71.1
	Sat_342	B2	20.31
	Sat_256	M	74.53
	Satt175	M	66.99
	Satt197	B1	46.39
	Satt481	L	54.57
	Sat_199	A2	84.09
	Satt301	D2	93.71
Skylla × PI437764	Satt691	E	19.7
	Satt153	O	118.4
	Satt243	O	119.5
	Sat_236	N	57.59
	Sat_342	B2	20.31
	Satt494	M	71.71
	Satt175	M	66.99

TABLE 1.10 (CONT'D)

	SSR markers	Linkage Groups	cM position
	Sat_092	D2	57.51
	Satt154	D2	57.07
	Satt475	K	78.68
	Satt197	B1	46.39
	Sat_340	L	55.51
	Satt301	D2	93.71
	Satt598	E	34.2
Skylla × PI548404	Satt197	B1	46.39
	Sat_247	B1	49.73
	Sat_340	L	55.51
	Satt154	D2	57.07
	Sat_234	F	66.55
	Sat_092	D2	57.51
	Satt494	M	71.71
	Sat_342	B2	20.31
	Sat_236	N	57.59
	Satt243	O	119.5
	Satt153	O	118.4
	Satt691	E	19.7

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CHAPTER TWO

GREENHOUSE SCREENING OF SOYBEAN GENOTYPES AND PLANT INTRODUCTIONS FOR RESISTANCE TO SCLEROTINIA STEM ROT

ABSTRACT

Two related but independent studies were conducted in the greenhouse. In the first study, 66 soybean plant introductions (PIs) were evaluated in the greenhouse for resistance to Sclerotinia stem rot in the winter of 2008 and 2009. All the 66 PIs, which were selected from more than six thousands PIs, were inoculated with *S. sclerotiorum* mycelia by drop-mycelium method. All the 66 PIs showed significant ($p < 0.005$) differences between lines for resistance to Sclerotinia stem rot. In the second study, 35 soybean genotypes were evaluated in the greenhouse and field conditions to predict the resistance levels of the lines for resistance to Sclerotinia stem rot. Greenhouse and field data had strong correlations with field data for resistance to Sclerotinia stem rot. They also showed different levels of resistance to Sclerotinia stem rot both in the field and the greenhouse studies. The data from drop-mycelium method of inoculation showed strong correlation of 0.63 ($P < 0.0005$) and 0.40 ($P< 0.0300$) with the data from spray-mycelium and Iowa field data respectively. This study showed that drop- and spray-mycelium methods are viable greenhouse methods to predict the field reaction of soybean to *S. sclerotiorum* infection.

INTRODUCTION

Sclerotinia stem rot of soybean caused by *Sclerotinia sclerotiorum* (Lib) de Bary is a major soybean disease in north-central regions of the United States and southern Canada. The disease caused the total yield loss of 59,275,000 bushels in the United States in 2009, which ranked after Soybean cyst nematode (http://www.aes.missouri.edu/delta/research/soyloss.stm) in terms of total yield loss in soybean. Yang et al. (1999) estimated yield loss of soybean due to Sclerotinia stem rot ranging from 170 to 335 kg ha^{-1} for each 10% increase in disease incidence.

S. sclerotiorum overwinters in the soil and debris as sclerotial bodies (Yang, 1997). The Sclerotinia stem rot was more prevalent when yearly temperatures were below normal (60-70°F) than when they were above normal. The prevalence of disease was less in no-till than in minimum-till fields (Workneh and Yang, 2000). The sporadic occurrence of Sclerotinia stem rot in soybean is due to the sensitivity of *S. sclerotiorum* to environmental factors. The soybean shows environmental-sensitivity to *S. sclerotiorum* pathogen. The response of soybean to *S. sclerotiorum* was studied with respect to light intensity and temperature in the greenhouse. Light-sensitive cultivars had decreased disease ratings as the photosynthetically active radiation (PAR) increased but light-insensitive cultivars had constant disease ratings with increased PAR (Pennypacker and Risium, 1999).

Different management practices have been applied to reduce the yield loss by Sclerotinia stem rot in soybean. Lactofen-treated soybean plants showed significant lesion size reduction when *S. sclerotiorum* were inoculated at V3 or R1 growth stages (Dann et al., 1999). Soybean tolerance to Sclerotinia stem rot was not related to glyphosate-resistance in soybean cultivars: 'S12-49', 'S14-M7' Roundup Ready ® (RR), 'S19-90', and 'S20-B9' (RR). Glyphosate did not

affect soybean growth and development or the incidence of Sclerotinia stem rot in glyphosate-resistant soybean (Nelson, 2000). Totir (2000) studied the effectiveness of seed treatment fungicides for controlling seed borne infections of soybean by *S. sclerotiorum*. Carboxim + thiram fungicides inoculated seeds showed reduced expression of fungus by 99% and PCNB + thiabendazole inoculated seeds showed 89% reduction in fungus expression.

Biological control agents such as *Pseudomonas chlororaphis, Bacillus amyloliquefaciens,* and *Pseudomonas* species have been used to control *S. sclerotiorum* both in greenhouse and field conditions in canola (Fernando et al., 2007). Similarly, Zeng et al. (2008) studied the effectiveness of biocontrol agents like *Coniothyrium minitans*, *Bacillus subtilis*, and *Trichoderma harzianum* to control *S. sclerotiorum* in soybean in controlled and field conditions. *C. minitans* and *T. harzianum* but *B. subtilis* significantly reduced the number and viability of sclerotia in both conditions. In other study, *Sporidesmium sclerotivorum* was used as biocontrol agent to determine its effectiveness in controlling Sclerotinia stem rot in soybean. Soybean plants were infested with macro conidia of *S. sclerotivorum* at a rate of 0, 2, 20, and 100 spores per cm^2. Plots infested with 20 and 100 spores per cm^2 had 56 to 100 percent less disease than control plots (del Rio et al, 2001). Soybean Sclerotinia stem rot was significantly reduced by the application of *B. subtilis* under control conditions. But the effectiveness of the biocontrol agent decreases if applied after 24 hours of *S. sclerotiorum* inoculation (Zhang and Xue, 2010).

Use of partial resistant varieties is the most effective method for controlling Sclerotinia stem rot in soybean (Kurle et al., 2001). Controlled environment screening is required to identify soybean cultivars that are partially resistant to Sclerotinia stem rot. But using greenhouse evaluation methods to determine the field response of soybean cultivars has been difficult. There

have been several studies carried out to predict the field response of soybean lines from the greenhouse and laboratory evaluation methods such as excised stem or detached leaf assay (Chun et al., 1987; Kull et al., 2003; Nelson et al.,1991; Wegulo et al., 1997), or cut stem inoculation method (Vuong and Hartman, 2003, and Kull et al., 2003). These methods are very time-consuming and tedious to carry out and results between greenhouse and field evaluations were poorly correlated (Kim et al., 2000; Nelson et al., 1991; Boland et al., 1987; Chun et al., 1987). Differences in reaction to Sclerotinia stem rot were reported among soybean cultivars (McLaren and Craven, 2008). Otto-Hanson et al. (2009) screened soybean germplasm for resistance to *S. sclerotiorum* by different inoculation methods. Instead of using F-test or root mean square error or coefficient of variation, sensitivity ratio was used to compare the power of plant and pathogen screening tests. Nelson et al. (1991) concluded that excised stem technique performed in laboratory for screening commercial soybean cultivars did not show any correlation with field data. The reaction data from cut stem inoculation method showed significant correlation ($P < 0.05$) with field data (Vuong et al., 2003). Cut stem inoculation method showed better result than detached-leaf and cotyledon methods when soybean and dry bean were screened for resistance to *S. sclerotiorum* in controlled environments (Kull et al., 2003). Ten cultivars of soybean were inoculated with *S. sclerotiorum* in laboratory and the disease reaction data were correlated with that of field data. The correlation coefficients between laboratory and field data varied in accordance with the inoculation methods used in laboratory for screening (Chun et al., 1987). Wegulo et al. (1998) studied different inoculation methods for screening soybean cultivars for resistance to *S. sclerotiorum* both in controlled and field environments. There were varied correlation coefficients between the data from controlled and field environments. Teran and Singh (2009) studied the efficacy of three greenhouse screening

methods for identifying physiological resistance to Sclerotinia stem rot in dry bean. Cut stem and infected bean flower methods were the most effective to identify physiological resistance to Sclerotinia stem rot in dry bean.

Drop- and spray- mycelium methods are the two convenient methods of evaluating soybean lines in the greenhouse conditions that predict the soybean reactions in the field conditions for resistance to Sclerotinia stem rot. In addition, these methods are cost effective, less time consuming, reliable, and convenient for large-scale evaluations (Chen and Wang, 2005).

Plant introductions (PIs) are important sources of genetic resistance to disease and pests. The narrow genetic-base of soybeans in the United States is due to the limited use of PIs in cultivar development. Only about eleven PIs were the major sources of current soybean cultivars (NAS, 1972; Gizlice et al., 1993). Shoener and Fehr (1978) argue that crosses involving PIs generally do not produce high yielding cultivars, though they are major sources of pest resistance genes. Thorne and Fehr (1970) discovered that the frequency of superior lines is greater in soybean populations derived from seventy-five percent adapted germplasm and twenty-five percent PIs. Exotic parentage needs to be introgressed with elite parentage to get high yield potential (Thompson and Nelson, 1998; Cornelious and Sneller, 2002; Vello et al., 1984). Similarly, Sneller (1999) concludes that soybean breeding is directed by extensive use of the parents derived from diverse crosses, which lead to significant yield increases. Narvel et al. (2000) found that the introgression of PIs germplasm into elite soybean cultivars depend on the amount of polymorphisms that exists between elite genotypes and PIs. Simple sequence repeat

(SSR) markers study showed that genetic diversity is more among the PIs than among the elite lines.

The objectives of this study were to a) screen 35 soybean genotypes for resistance to Sclerotinia stem rot in controlled (greenhouse) and field environments and assess the correlation among different inoculation methods and b) evaluate the 66 soybean plant introductions, which are partially resistant to Sclerotinia stem rot, using drop-mycelium method.

MATERIALS AND METHODS

Thirty-five soybean genotypes were chosen from different north-central soybean breeding programs based on the availability of their phenotypic data for reactions to Sclerotinia stem rot for this study (Table 2.1). Field experiments were carried out in Iowa and Wisconsin during the summer of 2004. The experiments were arranged in randomized complete block design (RCBD) with three replications for both locations. In Iowa, single row plots of 4.5-meter long were used. Corn was used as a wind barrier around the soybean field. The plants were inoculated with sorghum seeds infested with *S. sclerotiorum*. The misting system equipped with a sensor was used to maintain leaf wetness from the day of inoculation to the end of flowering. In Wisconsin, disease nursery plots had dimension of 5.9 × .38 m. Row spacing was 76 cm between outer and experimental rows. Common susceptible accession (Golden Harvest H2627RR) was planted in the two outer rows and an experimental accession was planted in the middle three rows. The plant canopy was almost complete when apothecia were applied. Air temperature was normal and rainfall was slightly above normal during most of the season. For field experiments, disease scoring was done according the disease severity index (DSI) described by Grau et al. (1982) at the R7 growth stage. Ten consecutive plants from each of the three

experimental rows were rated on a 0-4 scale: 0 = no symptoms; 1 = lesions on lateral branches only; 2 = lesions on main stem, no wilt, and normal pod development; 3 = lesions on main stem resulting in plant death and poor pod fill; 4 = lesions on main stem resulting in plant death and no yielding pods. A DSI was calculated as: 100 * [(sum of ratings for a plot)/ [5(number of ratings classes) * 30 (number of plants rated/plot)]].

The spray-mycelium method was carried out in December of 2009 at Michigan State University. The experimental design was a randomized complete block design with three replications and a minimum of 6 plants per line. Sterilized sclerotia were grown in potato dextrose agar medium and transferred into liquid potato dextrose broth. Potato dextrose broth was homogenized by constantly shaking in the shaker for four nights. The mycelium suspension was homogenized by blending in household blender. The blended mycelium suspension was sprayed on plants at the V3 growth stage. The inoculated plants were placed in plastic chambers and humidifiers were used to maintain a near 100% humidity inside the chambers. Approximately ten days after inoculation the total number of diseased plants were counted, and the percentage of plant mortality was calculated.

Similarly, the drop-mycelium method was performed as described by Chen and Wang (2005) for sixty-six PIs (Table 2.4). The pots were arranged as in spray-mycelium method, and mycelium suspension was also prepared as in spray-mycelium method. One ml of mycelium suspension was dropped on the top unfolding leaves of main stems. The plant mortality was calculated as described in spray-mycelium method.

The GLM procedure of SAS (SAS, 2008) was used to analyze the data from field and greenhouse experiments. Fisher's Protected Least Significant Differences (LSD) at a 5%

significance level was used to test the significance differences among genotypes in both greenhouse and field experiments. Replication 4 for 35 genotypes was not used for analysis purpose since the data had a lot of escapes (Table 2.1). Similarly, only two replications were used for analytical purpose in case of 66 PIs for the same purpose. Pearson's correlation coefficients between plant mortality of greenhouse and DSIs of the field experiments were calculated by the CORR procedure of SAS (Table 2.3). The broad-sense heritability was calculated using the same method used in Chapter 1.

RESULTS

All the genotypes inoculated in the greenhouse showed typical symptoms and signs of Sclerotinia stem rot (Fig. 2.1). The disease developed at multiple points in the plants for spray-mycelium method whereas disease progressed downward from apex for drop-mycelium method. In susceptible plants, disease progressed very fast but was arrested on the apical meristem in highly resistant lines. Necrotic lesions and white fluffy mycelia were visible on apical meristem and main stems. NKS-1990, a resistant check, reacted as expected, but BSR101, a susceptible check showed different level of resistance. AXN-1-68 consistently showed high level of resistance for different locations and evaluation methods while E99250 and LP02-240 consistently showed low level of resistance at all locations and for different evaluation methods (Table 2.2). Plant mortality of the genotypes evaluated in greenhouse ranged from 11.1% to 73% and 9.1% to 100% for drop-mycelium and spray-mycelium method, respectively. Data from 2009 spray-mycelium method is distorted because there was uneven spray of inocula (Fig. 2.3). Thus 2009 data were not included for calculating correlation. The DSI ranged from 20 to 94 and

from 25 to 72 for Wisconsin and Iowa, respectively. The correlation coefficient between the plant mortality from two greenhouse methods was 0.63 (*P< 0.0005*), implying that they are highly correlated (Table 2.3). The correlation coefficient between the DSI obtained from Iowa and the plant mortality obtained from drop and spray-mycelium method were 0.42 (*P< 0.03*) and 0.40 (*P<0.03*) respectively. In the field tests, correlation coefficient was 0.38 (*P < 0.05*) between Wisconsin and Iowa.

The 66 PIs showed different levels of resistance to *S. sclerotiorum.* The accessions; PI 506654, PI 506728, and PI 506733A, which belong to maturity group IV, showed consistently high level of resistance to *S. sclerotiorum* in both years. The accessions PI 189861, PI 417507, PI 548354, and PI 153316, which belong to maturity group 0, showed low level of resistance to *S. sclerotiorum for* both years. The other PIs did not show consistent result across years 2008 and 2009 in our study. The correlation coefficient between the plant mortality for 2008 and 2009 was 0.18.

Fig. 2.1 (a)

Fig. 2.1 (b)

FIGURE 2.1-SOYBEAN PLANTS BEFORE (A) AND AFTER (B) INOCULATION WITH *S. SCLEROTIORUM.*

DISCUSSION

An inoculation method is used to evaluate soybean germplasm for reaction to *S. sclerotiorum* in a controlled environment for two primary reasons. The reaction of soybean to Sclerotinia stem rot in the field nurseries can be inconsistent due to the quantitative mode of the partial resistance trait and unpredictable nature of the weather conditions for the same location in different years. True physiological resistance is difficult to identify because of disease escape in the field conditions. In case of the sixty-six PIs, those lines with higher partial resistance can be used as parents to develop partial resistance mapping populations and can be studied for QTLs associated with Sclerotinia stem rot resistance. In our study, data from drop-mycelium method showed high correlation (0.63) with spray-mycelium method, which were conducted in similar controlled conditions in different years. Chen and Wang (2005) argued that drop- and spray-mycelium methods are low-cost and high-efficiency greenhouse inoculation methods that give consistent and reproducible results. Our study validated that argument, and further argues that these methods can predict the field performance of soybean genotypes for resistance to Sclerotinia stem rot. The correlations between data from controlled environments with field performance ranged from 0.29 to 0.42, which signifies that for quantitative disease like Sclerotinia stem rot, these correlation coefficients are promising. Peltier and Grau (2008) found that Light intensity in controlled environments not only affect the Sclerotinia stem rot development in soybeans and but also affect the prediction of disease in field conditions. Since we controlled relative humidity and temperature inside the greenhouse, the intensity and duration of light hours might have some influence on the development of disease in the greenhouse.

Kim and Diers (2000) estimated the broad-sense heritability of Sclerotinia stem rot resistance at 0.59 in a 152 F3- derived lines from S19-90 crossed with Williams 82. The broad-

sense heritability for resistance to Sclerotinia stem rot ranged from 0.58 to 0.77 in common bean (Miklas and Grafton, 1992). Guo et al. (2008) estimated broad-sense heritability of 0.29 and 0.44 for $BC_1F_{4:5}$ and $BC_1F_{4:6}$ soybean lines, respectively. The broad-sense heritability for soybean PIs in our study were 0.58 and 0.62 for 2008 and 2009 greenhouse evaluations respectively, which closely agrees with Kim and Diers (2000). Our study approves the argument made by Grau et al. (1982) that resistance to Sclerotinia stem rot in soybean is a heritable trait.

The 66 plant introductions (PIs) were selected based on the fact that they showed partial resistance to Sclerotinia stem rot in different field locations and controlled environment conditions (Hoffman et al., 2002). Those PIs were evaluated in the greenhouse to narrow down to few PIs which would show promising resistance to Sclerotinia stem rot. Our study showed that those 66 PIs are significantly different ($P<0.0511$ and $P<$ 0.0182 for 2008 and 2009 evaluations, respectively) from each other for reactions to Sclerotinia stem rot (Table 2.5). Significantly different PIs based on least significant difference at $\alpha = 0.05$ is shown by Table 2.6. PI 427143, PI 506728, PI 506733A, PI 358318A, FC 030233, PI 132207, and PI 361059B showed consistently high level of resistance to Sclerotinia stem rot. These PIs range from early maturity (0) through late maturity group (IV). So these PIs can be used as sources of resistance in breeding for Sclerotinia stem rot resistance.

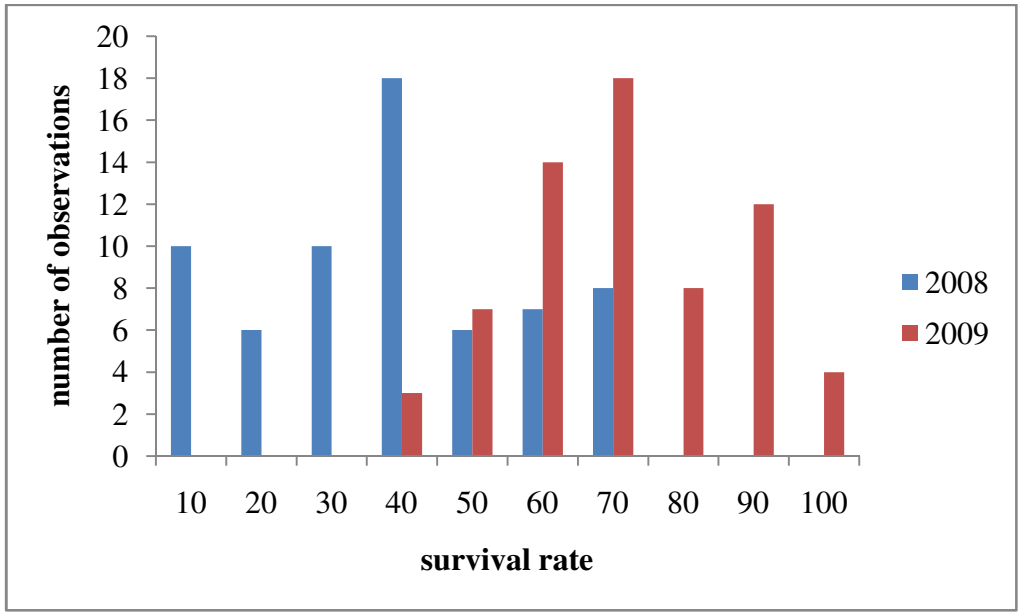

FIGURE 1.2- DIFFERENT LEVELS OF RESISTANCE SHOWN BY 66 PIS IN 2009 AND 2008 GREENHOUSE STUDY

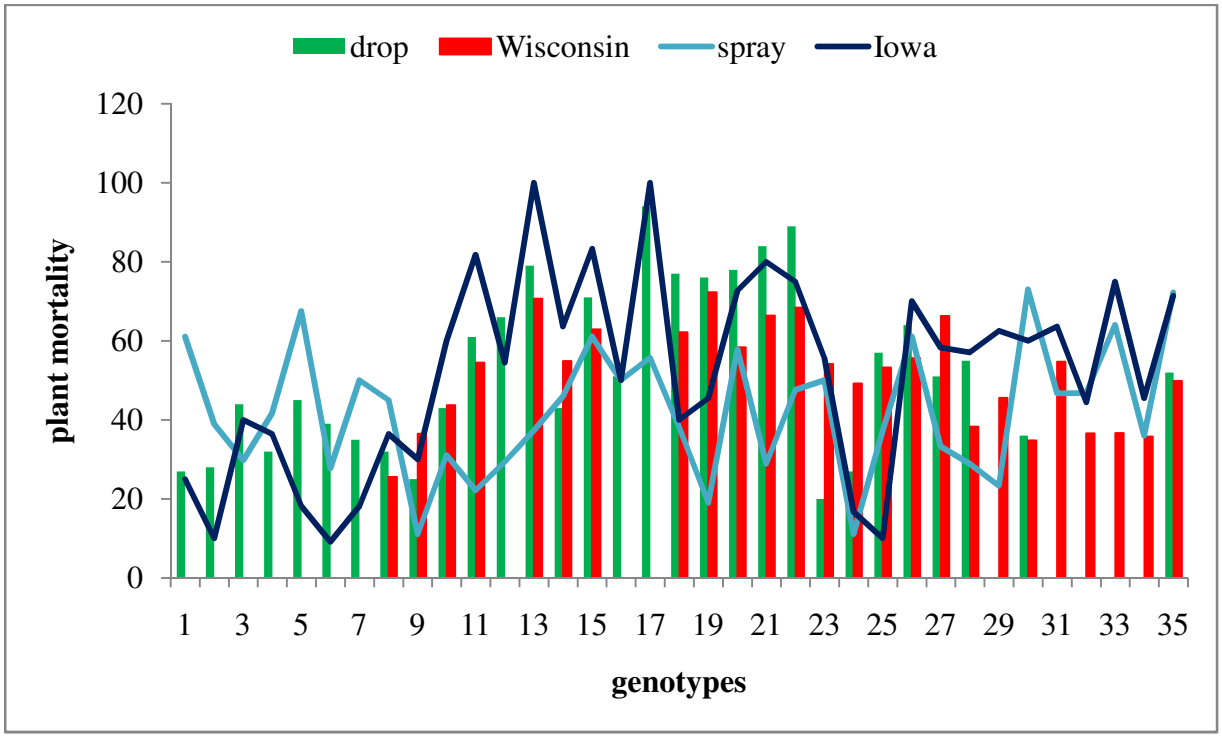

FIGURE 2.3- PLANT MORTALITY FOR 35 SOYBEAN GENOTYPES EVALUATED IN DIFFERENT ENVIRONMENTS

TABLE 2.1- THIRTY-FIVE SOYBEAN GENOTYPES AND THEIR REACTIONS TO SCLEROTINIA STEM ROT (SPRAY-MYCELIUM)

Genotypes	rep1	rep2	rep3	rep4	Mean mortality (%)
01SSD-106	83.0	83.0	80.0	0.0	61.5
01SSD-119	100.0	100.0	0.0	0.0	50.0
01SSD-150	50.0	50.0	25.0	20.0	36.3
01SSD-177	33.3	33.3	75.0	0.0	35.4
01SSD-20	50.0	50.0	85.7	0.0	46.4
01SSD-36	0.0	0.0	50.0	33.3	20.8
01SSD-61	85.7	85.7	50.0	0.0	55.4
AXN-1-55	60.0	60.0	25.0	0.0	36.3
AXN-1-68	33.3	33.3	0.0	16.7	20.8
AXN-2-55	60.0	60.0	33.3	60.0	53.3
A2506	66.7	66.7	0.0	0.0	33.3
BSR101	71.4	71.4	16.7	42.9	50.6
Dwight	50.0	50.0	28.6	83.3	53.0
E99279	66.7	66.7	0.0	14.3	36.9
HSO-3243	33.3	33.3	50.0	40.0	39.2

TABLE 2.1 (CONT'D)

Genotype	rep1	rep2	rep3	rep4	Mean mortality (%)
LD00-1938	50.0	50.0	0.0	50.0	37.5
LD00-497	50.0	50.0	57.1	0.0	39.3
LP02-221	71.4	71.4	42.9	66.7	63.1
LP02-222	57.1	57.1	0.0	66.7	45.2
LP02-240	57.1	57.1	116.7	66.7	74.4
LP02-250	66.7	66.7	0.0	71.4	51.2
LP02-253	83.3	83.3	16.7	25.0	52.1
Ohio FG3	100.0	100.0	0.0	42.9	60.7
NKS19-90	16.7	16.7	0.0	16.7	12.5
Skylla	83.0	83.0	0.0	0.0	41.5
U409006	100.0	100.0	66.7	0.0	66.7
U409014	16.7	16.7	83.3	100.0	54.2
U419020	66.7	66.7	20.0	50.0	50.8
U423040	20.0	20.0	50.0	100.0	47.5
U412014	85.7	85.7	83.3	85.7	85.1
NE3303	100.0	100.0	40.0	0.0	60.0

TABLE 2.2- REACTIONS OF 35 SOYBEAN GENOTYPES TO DIFFERENT METHOD OF INOCULATIONS

Genotypes	**Drop**[3]	**Iowa DSI**[4]	**WisconsinDSI**[4]	**Spray10**[3]
001SSD-106	25.0		27.0	61.0
01SSD-119	10.0		28.0	38.9
01SSD-150	40.0		44.0	29.8
01SSD-177	36.4		32.0	41.7
01SSD-20	18.2		45.0	67.5
01SSD-36	9.1		39.0	27.8
01SSD-61	18.2		35.0	50.0
AXN-1-55	36.4	25.6	32.0	45.0
AXN-1-68	30.0	36.5	25.0	11.1
AXN-2-55	60.0	43.7	43.0	31.1
A2506	81.8	54.5	61.0	22.2
BSR101	54.5		66.0	29.4
Dwight	100.0	70.7	79.0	37.3
E99279	63.6	54.9	43.0	46.0
HSO-3243	83.3	62.9	71.0	61.1
LD00-1938	50.0		51.0	50.0
LD00-497	100.0		94.0	55.7

[3] Greenhouse evaluation methods (plant mortality percentage)

[4] data from the fields (Disease Severe Index)

TABLE 2.2 (CONT'D)

Genotypes	Drop[3]	Iowa DSI[4]	Wisconsin DSI[4]	Spray10[3]
LP02-221	40.0	62.2	77.0	38.1
LP02-222	45.5	72.3	76.0	19.0
LP02-240	72.7	58.4	78.0	57.9
LP02-250	80.0	66.4	84.0	28.9
LP02-253	75.0	68.4	89.0	47.6
Ohio FG3	55.6	54.2	20.0	50.0
NKS19-90	16.7	49.2	27.0	11.1
Skylla	10.0	53.3	57.0	37.2
U409006	70.0	55.6	64.0	61.1
U409014	58.3	66.3	51.0	33.3
U419020	57.1	38.3	55.0	28.9
U423040	62.5	45.6		23.3
U412014	60.0	34.8	36.0	73.0
NE3303	63.6	54.7		46.7
U413038	44.4	36.6		46.8
U425043	75.0	36.7		64.0
U416019	45.5	35.8		36.0

TABLE 2.2 (CONT'D)

Genotypes	Drop[3]	Iowa DSI[4]	Wisconsin DSI[4]	Spray10[3]
E99250	71.4	49.9	52.0	72.2
Mean	**52.0**	**51.5**	**52.7**	**42.3**
STDEV	**24.9**	**12.9**	**21.1**	**16.4**
RMSE	**4.2**	**2.6**	**3.9**	**2.8**
LSD	**26.8**	**20.7**	**22.8**	**49.5**

TABLE 2.3- PEARSON CORRELATION COEFFICIENTS, N = 26 PROB > |R| UNDER H0: RHO=0, FOR 35 GENOTYPES

	Drop	**Iowa**	**Wisconsin**
Iowa	0.42073*(0.0323)*		
Wisconsin	0.30162*(0.1343)*	0.38577*(0.0516)*	
Spray10	0.63379*(0.0005)*	0.40883*(0.0381)*	0.29231*(0.1473)*

Correlations are followed by their respective *p*-values at $\alpha = 0.05$

TABLE 2.4- SIXTY-SIX SOYBEAN PIS AND THEIR REACTIONS TO SCLEROTINIA STEM ROT (DROP-MYCELIUM METHOD)

		Survival rate (%)				Survival rate (%)				Survival rate (%)	
PID	**PIs**	**2008**	**2009**	**PID**	**PIs**	**2008**	**2009**	**PID**	**PIs**	**2008**	**2009**
1	PI 132207	57.8	60.0	23	PI 091733	55.0	47.2	45	PI 507352	73.3	35.0
2	PI 153259	50.0	54.4	24	PI 153282	64.3	34.3	46	PI 507353	71.4	45.6
3	PI 189861	33.3	33.3	25	PI 153316	40.0	41.7	47	PI 196157	90.5	34.4
4	PI 189899	50.0	38.9	26	PI 184042	88.9	32.8	48	PI 229324	58.1	13.3
5	PI 232996	63.9	45.8	27	PI 189896	66.7	16.7	49	PI 398637	69.1	31.7
6	PI 243547	67.8	40.0	28	PI 189919	83.3	42.2	50	PI 404180	62.0	39.5
7	PI 291319B	36.1	24.4	29	PI 391589B	65.7	13.3	51	PI 417201	93.3	44.4
8	PI 361059B	81.1	58.9	30	PI 416776	66.7	41.7	52	PI 423818	61.1	0.0
9	PI 417449	70.0	11.1	31	PI 416805	91.7	36.1	53	PI 417245	73.3	16.7
10	PI 417507	30.0	25.0	32	PI 427143	68.9	58.3	54	PI 506519	33.3	49.4
11	PI 417533	33.3	50.0	33	PI 504502	94.4	62.2	55	PI 506652	73.5	30.0
12	PI 437072	42.1	27.8	34	PI 548312	62.2	35.6	56	PI 506654	94.4	52.8
13	PI 437527	46.8	16.7	35	PI 548380	58.3	31.1	57	PI 506728	77.1	70.0
14	PI 437764	54.0	23.6	36	PI 548407	75.6	34.4	58	PI 506733A	91.7	69.4

TABLE 2.4 (CONT'D)

		Survival rate (%)				Survival rate (%)				Survival rate (%)	
PID	**PIs**	**2008**	**2009**	**PID**	**PIs**	**2008**	**2009**	**PID**	**PIs**	**2008**	**2009**
16	PI 548354	37.2		38	PI 561284	70.0	5.6	60	PI 506868	66.7	16.7
17	PI 548404	58.3	33.3	39	PI 561331	45.2	13.3	61	PI 506892	90.5	24.4
18	PI 548539	72.2	47.2	40	PI 561345	47.8	50.0	62	PI 507222	73.8	50.0
19	PI 567157A	93.3	11.1	41	PI 561353	63.3	36.7	63	PI 567650B	66.7	18.9
20	PI 578501	50.0	38.9	42	PI 561367	86.7	25.0	64	PI 567721	36.7	5.6
21	FC 030233	73.3	58.3	43	PI 189931	40.0	33.3	65	PI 594286	100.0	35.5
22	PI 081775	64.7		44	PI 358318A	81.0	58.3	66	PI 594289	71.7	19.4
									Mean	**65.3**	**34.8**
									STDEV	**18.0**	**16.9**
									LSD		**46.3**

TABLE 2.5- SIGNIFICANT DIFFERENCES AMONG THE PLANT INTRODUCTIONS SCREENED FOR RESISTANCE TO SCLEROTINIA STEM ROT (2008 AND 2009 DATA, RESPECTIVELY)

Source	DF	Type III SS	Mean Square	F-Value	Pr > F
pid	65	69668. 37889	1071.82121	1.41	0.0511
pid	65	63756.53076	980.86970	1.68	0.0182

'pid' means plant ID

TABLE 2.6- SIGNIFICANCE DIFFERENCE BETWEEN PIS SHOWN BY LEAST SIGNIFICANT DIFFERENCE (LSD) TEST

t Grouping at α=0.05	Mean	pid (PI)
A	87.50	59 (PI 506784)
ab	75.00	33 (PI 504502)
abc	71.67	58 (PI 506733A)
bc	70.83	19 (PI 567157A)
abcd	63.33	9 (PI 417449)
abcdefg	39.29	51 (PI 417201)
cdefg	26.67	36 (PI 548407)
cdefg	25.00	28 (PI 189919)
efg	12.50	68 (PI 594289)
g	0.00	38 (PI 561284)
g	0.00	14 (PI 437764)

TABLE 2.7- BROAD-SENSE HERITABILITIES FOR 66 PIS EVALUATED IN 2008 AND 2009

Sources of variation	**Mean Square**	
	2008	**2009**
Genotype	1071.8212	980.86970
Error	762.1222	582.4342
Heritability	0.58	0.62

APPENDIX

TABLE A1- 66 PIS AND THEIR MATURITY GROUP, ORIGIN, AND SURVIVAL RATE FOR 2009 GREENHOUSE EVALUATION

Plant Introductions		Maturity group	Origin	rep 1	rep 2	rep 3	Mean survival (%)
WP02	PI 132207	0	Netherlands	80.0	0.0	100.0	60.0
WP03	PI 153259	0	Belgium	50.0	33.3	80.0	54.4
WP04	PI 189861	0	Germany	0.0	0.0	100.0	33.3
WP05	PI 189899	0	France	66.7	50.0	0.0	38.9
WP06	PI 232996	0	Germany	37.5	80.0	20.0	45.8
WP07	PI 243547	0	Japan	20.0	0.0	100.0	40.0
WP08	PI 291319B	0	China	33.3	0.0	40.0	24.4
WP09	PI 361059B	0	China	60.0	66.7	50.0	58.9
WP10	PI 417449	0	Japan	0.0	0.0	33.3	11.1
WP11	PI 417507	0	Germany	25.0	50.0	0.0	25.0
WP12	PI 417533	0	Germany	50.0	50.0	50.0	50.0
Wp13	PI 437072	0	Russian federation	50.0	0.0	33.0	27.7
WP14	PI 437527	0	Ukraine	0.0	0.0	50.0	16.7
WP15	PI 437764	0	China	14.3	40.0	16.7	23.6
WP16	PI 438267	0	China	0.0	100.0	33.3	44.4
WP17	PI 548354	0	China	0.0	0.0	0.0	0.0

TABLE A1 (CONT'D)

Plant Introductions		Maturity group	Origin	rep 1	rep 2	rep 3	Mean survival (%)
WP19	PI 548539	0	Canada	66.7	75.0	0.0	47.2
WP20	PI567157A	0	China	0.0	33.3	0.0	11.1
WP21	PI 578501	0	China	0.0	16.7	100.0	38.9
WP22	FC 030233	I	Canada	50.0	25.0	100.0	58.3
WP23	PI 081775	I	Japan	33.3	20.0	80.0	44.4
WP24	PI 091733	I	China	25.0	50.0	66.7	47.2
WP25	PI 153282	I	Belgium	60.0	42.9	0.0	34.3
WP26	PI 153316	I	France	25.0	0.0	100.0	41.7
WP27	PI 184042	I	Yugoslavia	40.0	33.3	25.0	32.8
WP28	PI 189896	I	Germany	0.0	50.0	0.0	16.7
WP29	PI 189919	I	France	60.0	0.0	66.7	42.2
WP30	PI 391589B	I	China	0.0	0.0	40.0	13.3
WP31	PI 416776	I	Japan	100.0	25.0	0.0	41.7
WP32	PI 416805	I	Japan	33.3	25.0	50.0	36.1

TABLE A1 (CONT'D)

Plant Introductions		Maturity group	Origin	rep 1	rep 2	rep 3	Mean survival (%)
WP33	PI 427143	I	South Korea	50.0	100.0	25.0	58.3
WP34	PI 504502	I	Taiwan	20.0	66.7	100.0	62.2
WP35	PI 548312	I	China	50.0	16.7		33.3
WP36	PI 548380	I	China	20.0	33.3	40.0	31.1
WP37	PI 548407	I	Japan	20.0	16.7	66.7	34.4
WP38	PI 549066	I	Japan	0.0	0.0	75.0	25.0
WP39	PI 561284	I	China	0.0	16.7	0.0	5.6
WP40	PI 561331	I	China	20.0	0.0	20.0	13.3
WP41	PI 561345	I	China	16.7	33.3	100.0	50.0
WP42	PI 561353	I	China	60.0	0.0	50.0	36.7
WP43	PI 561367	I	China	0.0	25.0	50.0	25.0
WP44	PI 189931	II	France	0.0	50.0	50.0	33.3
WP45	PI358318A	II	Japan	100.0	25.0	50.0	58.3
WP46	PI 507352	II	Japan	25.0	80.0	0.0	35.0
WP47	PI 507353	II	Japan	16.7	80.0	40.0	45.6
WP48	PI 196157	III	Japan	20.0	0.0	83.3	34.4

TABLE A1 (CONT'D)

Plant Introductions		Maturity group	Origin	rep 1	rep 2	rep 3	Mean survival (%)
WP54	PI 417245	IV	Japan	0.0	0.0	50.0	16.7
WP55	PI 506519	IV	Japan	33.3	40.0	75.0	49.4
WP56	PI 506652	IV	Japan	40.0	50.0	0.0	30.0
WP57	PI 506654	IV	Japan	25.0	50.0	83.3	52.8
WP58	PI 506728	IV	Japan	60.0	83.3	66.7	70.0
WP59	PI506733A	IV	Japan	100.0	75.0	33.3	69.4
WP60	PI 506784	IV	Japan	0.0	0.0	50.0	16.7
WP61	PI 506868	IV	Japan	50.0	0.0	0.0	16.7
WP62	PI 506892	IV	Japan	0.0	40.0	33.3	24.4
WP63	PI 507222	IV	Japan	16.7	33.3	100.0	50.0
WP65	PI567650B	IV	China	16.7	0.0	40.0	18.9
WP66	PI 567721	IV	China	0.0	0.0	16.6	5.5
WP67	PI 594286	IV	Japan	0.0	40.0	66.6	35.5
WP68	PI 594289	IV	Japan	0.0	25.0	33.3	19.4

TABLE A2- SIXTY-SIX SOYBEAN PIS AND THEIR MATURITY GROUPS, ORIGINS, AND REACTIONS TO SCLEROTINIA STEM ROT EVALUATED IN 2008

Test Number	PIs	Maturity Group	Origin	rep1	rep2	rep3	Mean Survival (%)
WP02	PI132007	0	Netherlands	100.0	40.0	33.3	57.8
WP03	PI 153259	0	Belgium	100.0	0.0	50.0	50.0
WP04	PI 189861	0	Germany	50.0	33.3	16.7	33.3
WP05	PI 189899	0	France	50.0	50.0	60.0	50.0
WP06	PI 232996	0	Germany	100.0	16.7	75.0	63.9
WP07	PI 243547	0	Japan	83.3	80.0	40.0	67.8
WP08	PI 291319B	0	China	16.7	16.7	75.0	36.1
WP09	PI 361059B	0	China	100.0	60.0	83.3	81.1
WP10	PI 417449	0	Japan	100.0	50.0	60.0	70.0
WP11	PI 417507	0	Germany	40.0	50.0	0.0	30.0
WP12	PI 417533	0	Germany	0.0	50.0	50.0	33.3
WP13	PI 437072	0	Russian Federation	16.7	42.9	66.7	42.1
WP14	PI 437527	0	Ukraine	50.0	57.2	33.3	46.8
WP15	PI 437764	0	China	33.3	28.6	100.0	54.0
WP16	PI 438267	0	China	80.0	80.0	33.3	64.4
WP17	PI 548354	0	China	66.7	25.0	20.0	37.2
WP18	PI 548404	0	Canada	75.0	25.0	75.0	58.3
WP19	PI 548539	0	Canada	66.7	75.0	75.0	72.2

TABLE A2 (CONT'D)

Test Number	PIs	Maturity Group	Origin	Rep1	Rep2	Rep3	Mean Survival (%)
WP22	FC 030233	I	Canada	20.0	100.0	100.0	73.3
WP23	PI 081775	I	Japan	25.0	83.3	85.7	64.7
WP24	PI 091733	I	China	40.0	50.0	75.0	55.0
WP25	PI 153282	I	Belgium	57.2	85.7	50.0	64.3
WP26	PI 153316	I	France	0.0	100.0	20.0	40.0
WP27	PI 184042	I	Yugoslavia	83.3	100.0	83.3	88.9
WP28	PI 189896	I	Germany	60.0	80.0	60.0	66.7
WP29	PI 189919	I	France		83.3		83.3
WP30	PI 391589B	I	China	57.2	60.0	80.0	65.7
WP31	PI 416776	I	Japan	50.0	66.7	83.3	66.7
WP32	PI 416805	I	Japan	100.0	75.0	100.0	91.7
WP33	PI 427143	I	South Korea	80.0	66.7	60.0	68.9
WP34	PI 504502	I	Taiwan	100.0	83.3	100.0	94.4
WP35	PI 548312	I	China	66.7	100.0	20.0	62.2
WP36	PI 548380	I	China	100.0	75.0	0.0	58.3
WP37	PI 548407	I	Japan	66.7	60.0	100.0	75.6
WP38	PI 549066	I	Japan	100.0	83.3	60.0	81.1
WP39	PI 561284	I	China	60.0	75.0	75.0	70.0
WP40	PI 561331	I	China	85.7	16.7	33.3	45.2
WP41	PI 561345	I	China	50.0	33.3	60.0	47.8

TABLE A2 (CONT'D)

Test Number	PIs	Maturity Group	Origin	rep1	rep2	rep3	Mean survival (%)
WP43	PI 561367	I	China	100.0	60.0	100.0	86.7
WP44	PI 189931	II	France	33.0	20.0	0.0	40.0
WP45	PI 358318A	II	Japan	100.0	100.0	42.9	81.0
WP46	PI 507352	II	Japan	40.0	100.0	80.0	73.3
WP47	PI 507353	II	Japan	100.0	57.2	57.2	71.4
WP48	PI 196157	III	Japan	71.4	100.0	100.0	90.5
WP49	PI 229324	III	Japan	14.3	100.0	60.0	58.1
WP50	PI 398637	III	South Korea	57.2	50.0	100.0	69.1
WP51	PI 404180	III	China	16.7	85.7	83.3	62.0
WP52	PI 417201	III	Japan	100.0	80.0	100.0	93.3
WP53	PI 423818	III	South Korea	50.0	50.0	83.3	61.1
WP54	PI 417245	IV	Japan	20.0	100.0	100.0	73.3
WP55	PI 506519	IV	Japan	0.0	0.0	100.0	33.3
WP56	PI 506652	IV	Japan	83.3	57.2	80.0	73.5
WP57	PI 506654	IV	Japan	83.3	100.0	100.0	94.4
WP58	PI 506728	IV	Japan	60.0	100.0	71.4	77.1
WP59	PI 506733A	IV	Japan	100.0	75.0	100.0	91.7
WP60	PI 506784	IV	Japan	83.3	100.0	100.0	94.4
WP61	PI 506868	IV	Japan	57.1	42.9	100.0	66.7
WP62	PI 506892	IV	Japan	100.0	71.4	100.0	90.5

TABLE A2 (CONT'D)

Test Number	PIs	Maturity Group	Origin	rep1	rep2	rep3	Mean survival (%)
WP65	PI 567650B	IV	China	66.7	50.0	83.3	66.7
WP66	PI 567721	IV	China	50.0	0.0	100.0	36.7
WP67	PI 594286	IV	Japan	100.0	100.0	60.0	100.0
WP68	PI 594289	IV	Japan	75.0	100.0	40.0	71.7
Checks	**S19-90**			75.0		100.0	87.5
	Olympus			33.3		33.3	33.3

TABLE A3 PLANT MORTALITY FOR 392 LINES EVALUATED BY TWO DIFFERENT METHODS

		Plant Mortality (%)				Plant Mortality (%)	
Plant ID	**Population**	**spray**	**Drop**	**Plant ID**	**Population**	**spray**	**Drop**
1	1	67.0	55.6	197	4	33.3	70.8
2	1	73.0	22.2	198	4	29.2	52.4
3	1	58.2	35.7	199	4	21.2	75.9
4	1	77.6	36.7	200	4	54.2	14.3
5	1	79.2	38.9	201	4	30.3	66.7
6	1	63.1	55.6	202	4	40.0	65.5
7	1	67.9	60.0	203	4	45.5	60.7
8	1	80.8	84.1	204	4	29.2	45.8
9	1	69.1	23.8	205	4	12.5	70.8
10	1	91.3	50.0	206	4	25.3	55.7
11	1	91.3	24.5	207	4	34.1	30.6
12	1	74.3	0.0	208	4	8.3	50.5
13	1	72.9	11.1	209	4	36.3	63.9
14	1	81.3	33.3	210	4	47.5	41.7
15	1	88.6	0.0	211	4	4.2	54.6
16	1	75.0	5.6	212	4	34.1	72.2
17	1	75.6	5.6	213	4	50.7	33.3
18	1	81.6	18.9	214	4	50.0	26.1
19	1	75.0	16.7	215	4	16.7	33.3
20	1	78.4	0.0	216	4	41.7	62.5
21	1	100.0	8.3	217	4	54.2	57.1
22	1	9.5	9.5	218	4	38.1	79.4
23	1	14.2	0.0	219	4	53.6	54.2

TABLE A3 (CONT'D)

Plant ID	Population	Plant Mortality (%) Spray	Drop	Plant ID	Population	Plant Mortality (%) Spray	Drop
24	1	25.8	0.0	220	4	35.2	36.7
25	1	76.1	22.2	221	4	40.0	72.2
26	1	86.0	10.3	222	4	60.0	46.7
27	1	73.3	11.1	223	4	50.0	22.2
28	1	84.6	6.7	224	4	54.8	29.1
29	1	68.3	33.3	225	4	52.5	40.0
30	1	85.0	0.0	226	4	32.3	33.3
31	1	0.0	42.9	227	4	44.3	72.2
32	1	75.7	27.8	228	4	43.3	83.3
33	1	81.3	15.1	229	4	25.0	73.6
34	1	82.0	16.7	230	4	100.0	91.7
35	1	78.4	0.0	231	4	45.5	68.1
36	1	72.7	11.1	232	4	65.3	60.0
37	1	48.9	26.7	233	4	64.0	48.2
38	1	12.7	0.0	234	4	9.1	35.7
39	1	76.3	11.4	235	5	47.0	65.1
40	1	77.5	40.0	236	5	52.8	48.9
41	1	95.8	22.2	237	5	41.7	66.7
42	1	85.3	0.0	238	5	47.3	57.1
43	1	67.6	0.0	239	5	37.5	52.8
44	1	72.1	9.5	240	5	36.0	25.6
45	1	62.5	48.9	241	5	8.3	55.6
46	1	88.6	19.1	242	5	70.8	45.6
47	1	80.9	20.0	243	5	56.1	22.2
48	1	87.5	16.7	244	5	50.0	54.4
49	1	89.6	24.6	245	5	39.1	41.1
50	1	76.9	0.0	246	5	68.2	67.8

TABLE A3 (CONT'D)

Plant ID	Population	Plant Mortality (%) Spray	Drop	Plant ID	Population	Plant Mortality (%) Spray	Drop
51	1	86.7	0.0	247	5	63.6	33.3
52	1	79.7	0.0	248	5	60.7	17.8
53	1	54.7	0.0	249	5	18.2	42.2
54	1	86.4	6.7	250	5	12.5	53.3
55	1	81.3	0.0	251	5	45.5	41.7
56	1	95.4	22.2	252	5	12.5	48.4
57	1	48.9	11.1	253	5	34.8	23.3
58	1	70.6	0.0	254	5	28.6	50.0
59	1	80.3	33.3	255	5	50.0	50.8
60	2	81.7	6.7	256	5	45.8	26.7
61	2	51.7	72.2	257	5	29.2	5.6
62	2	70.1	22.2	258	5	31.1	17.8
63	2	72.9	27.8	259	5	45.8	11.1
64	2	91.3	41.1	260	5	87.5	16.7
65	2	93.8	58.3	261	5	45.8	11.1
66	2	44.3	63.3	262	5	47.0	18.9
67	2	100.0	35.6	263	5	52.7	61.1
68	2	14.3	30.6	264	5	52.8	25.0
69	2	13.4	53.3	265	5	30.6	30.0
70	2	0.0	51.1	266	5	51.5	61.1
71	2	45.0	50.0	267	5	31.3	32.9
72	2	51.2	66.7	268	5	43.2	34.4
73	2	86.8	46.7	269	5	56.8	44.4
74	2	32.2	36.7	270	5	68.2	18.7
75	2	100.0	41.7	271	5	50.0	65.6
76	2	76.1	18.9	272	5	41.4	25.0

TABLE A3 (CONT'D)

Plant ID	Population	Spray	Plant Mortality (%) Drop	Plant ID	Population	Spray	Plant Mortality (%) Drop
77	2	79.0	25.6	273	5	33.3	25.0
78	2	23.3	40.0	274	5	42.4	19.8
79	2	33.3	38.9	275	5	26.7	58.9
80	2	29.2	11.1	276	5	58.3	22.2
81	2	31.8	16.7	277	5	0.0	43.3
82	2	48.1	66.7	278	5	21.0	41.1
83	2	83.2	34.4	279	5	34.8	30.0
84	2	20.8	21.7	280	5	41.1	44.4
85	2	81.8	27.8	281	5	56.8	22.2
86	2	27.7	63.3	282	5	63.6	55.6
87	2	91.6	34.4	283	5	50.0	42.2
88	2	63.0	55.6	284	5	40.0	38.9
89	2	69.2	61.1	285	5	11.1	28.6
90	2	45.0	25.6	286	6	61.0	30.6
91	2	66.3	40.0	287	6	68.9	8.3
92	2	43.6	13.3	288	6	40.1	13.3
93	2	68.4	28.1	289	6	16.7	47.8
94	2	75.9	22.2	290	6	17.8	43.9
95	2	40.7	38.9	291	6	43.3	50.0
96	2	48.6	33.3	292	6	14.3	25.0
97	2	42.3	38.9	293	6	18.2	27.6
98	2	34.9	38.9	294	6	35.6	21.7
99	2	45.5	42.8	295	6	30.3	66.1
100	2	72.7	36.1	296	6	50.0	33.3
101	2	26.7	41.7	297	6	61.4	74.3
102	2	76.9	68.9	298	6	67.7	80.6

TABLE A3 (CONT'D)

Plant ID	Population	Plant Mortality (%) Spray	Drop	Plant ID	Population	Plant Mortality (%) Spray	Drop
103	2	40.9	11.1	299	6	23.2	66.7
104	2	39.3	30.0	300	6	21.2	66.7
105	2	15.0	27.8	301	6	27.3	33.3
106	2	34.1	38.9	302	6	21.6	61.1
107	2	40.0	26.7	303	6	33.3	58.7
108	2	25.0	38.9	304	6	58.3	43.3
109	2	45.5	46.7	305	6	32.5	70.2
110	3	77.1	49.6	306	6	37.5	72.7
111	3	79.9	74.6	307	6	91.7	77.8
112	3	93.6	62.7	308	6	29.2	53.3
113	3	88.9	45.6	309	6	25.0	69.1
114	3	88.5	36.0	310	6	32.5	83.0
115	3	66.7	61.1	311	6	32.6	50.0
116	3	57.5	66.7	312	6	83.3	68.9
117	3	78.8	60.2	313	6	50.0	87.5
118	3	85.4	44.4	314	6	14.5	68.9
119	3	72.5	55.0	315	6	50.0	58.9
120	3	89.6	49.7	316	6	51.4	80.0
121	3	81.7	44.4	317	6	4.5	73.3
122	3	82.1	88.9	318	6	46.4	72.2
123	3	64.6	46.0	319	6	48.6	61.1
124	3	88.8	11.1	320	6	37.5	77.8
125	3	90.0	18.9	321	6	42.5	75.0
126	3	87.1	59.0	322	6	40.0	59.1
127	3	57.5	47.8	323	6	62.8	70.5
128	3	75.0	68.9	324	7	48.5	42.1

TABLE A3 (CONT'D)

Plant ID	Population	Plant Mortality (%) Spray	Drop	Plant ID	Population	Plant Mortality (%) Spray	Drop
129	3	42.3	48.0	325	7	13.6	52.4
130	3	69.7	46.3	326	7	46.1	30.2
131	3	83.6	26.2	327	7	37.2	16.7
132	3	87.1	25.0	328	7	58.3	0.0
133	3	86.9	33.3	329	7	36.0	19.1
134	3	23.3	66.7	330	7	26.1	37.8
135	3	84.5	56.7	331	7	52.0	0.0
136	3	90.8	16.7	332	7	47.0	15.9
137	3	42.9	25.0	333	7	14.5	44.1
138	3	88.0	81.5	334	7	41.7	65.5
139	3	74.4	47.5	335	7	63.6	26.2
140	3	77.7	55.4	336	7	13.9	46.7
141	3	97.9	49.5	337	7	29.2	5.6
142	3	46.7	58.3	338	7	12.5	0.0
143	3	83.0	36.1	339	7	49.6	9.7
144	3	72.7	38.4	340	7	39.5	33.3
145	3	83.0	54.4	341	7	44.4	39.0
146	3	87.5	37.8	342	7	32.7	50.0
147	3	70.9	8.3	343	7	47.3	68.9
148	3	80.9	70.2	344	7	37.5	16.3
149	3	86.6	48.5	345	7	51.5	4.8
150	3	90.2	31.7	346	7	52.7	20.0
151	3	72.9	51.6	347	7	12.5	50.0
152	3	83.4	56.3	348	7	16.1	11.1
153	3	91.7	31.7	349	7	25.0	0.0
154	3	93.2	36.9	350	7	18.2	34.5

TABLE A3 (CONT'D)

		Plant Mortality (%)				Plant Mortality (%)	
Plant ID	**Population**	**Spray**	**Drop**	**Plant ID**	**Population**	**Spray**	**Drop**
155	3	87.5	33.3	351	7	34.1	30.7
156	3	90.1	62.4	352	7	47.3	67.8
157	3	93.8	47.8	353	7	23.7	44.5
158	3	95.5	33.3	354	7	15.6	39.0
159	3	80.7	58.2	355	7	5.6	0.0
160	3	87.5	63.5	356	7	36.4	0.0
161	3	78.1	40.0	357	7	18.6	6.7
162	3	56.1	40.7	358	7	48.2	5.7
163	3	97.9	51.9	359	7	41.2	95.2
164	3	83.3	80.0	360	7	14.9	38.1
165	3	81.9	44.4	361	7	19.4	93.3
166	3	86.7	51.9	362	7	60.2	41.7
167	3	97.9	29.3	363	7	4.2	5.7
168	3	90.9	33.3	364	7	31.8	16.7
169	3	68.6	65.5	365	7	31.1	16.7
170	3	80.7	72.2	366	7	18.2	20.6
171	3	85.7	76.4	367	7	25.5	5.6
172	3	95.5	31.7	368	7	17.7	5.7
173	4	65.0	68.1	369	7	14.1	44.6
174	4	37.5	66.1	370	7	4.5	57.2
175	4	31.8	56.6	371	7	22.7	34.2
176	4	9.1	70.7	372	7	37.8	0.0
177	4	25.4	62.7	373	7	38.2	13.3
178	4	81.9	53.3	374	7	25.4	59.1
179	4	87.5	59.1	375	7	0.0	0.0
180	4	78.5	22.2	376	7	18.2	41.7

TABLE A3 (CONT'D)

		Plant Mortality (%)				Plant Mortality (%)	
Plant ID	**Population**	**Spray**	**Drop**	**Plant ID**	**Population**	**Spray**	**Drop**
181	4	58.9	33.3	377	7	32.5	16.7
182	4	61.9	46.2	378	7	39.4	33.4
183	4	74.2	25.0	379	7	19.4	52.4
184	4	43.2	33.3	380	7	54.2	22.2
185	4	86.9	83.3	381	7	13.3	40.0
186	4	88.5	72.2	382	7	12.5	11.1
187	4	81.3	79.1	383	7	25.8	38.9
188	4	10.0	63.0	384	7	20.0	21.0
190	4	58.3	22.2	386	7	11.1	26.4
191	4	50.0	45.6	387	7	35.0	63.3
192	4	22.0	35.1	388	7	17.0	33.3
193	4	32.1	69.1	389	7	44.5	59.6
194	4	9.1	33.3	390	7	29.2	9.7
195	4	30.7	31.6	391	7	24.3	38.1
196	4	21.4	33.4	392	7	0.0	38.1
				NKS19-90		**74.4**	**27.1**
				Olympus			**43.5**
				Mean		**51.9**	**39.6**
				STDEV		**26.3**	**22.4**

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