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GENETIC MAPPING OF QTLs UNDERLYING PARTIAL SCLEROTINIA STEM ROT RESISTANCE IN SOYBEAN

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GENETIC MAPPING OF QTLs UNDERLYING PARTIAL SCLEROTINIA STEM ROT RESISTANCE IN SOYBEAN

By

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ABSTRACT

GENETIC MAPPING OF QTLs UNDERLYING PARTIAL SCLEROTINIA STEM ROT RESISTANCE IN SOYBEAN

By

Xiaomei Guo

Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, is an important yield reducing disease of soybean in the Midwestern states of the U.S. The objective of my study was to identify quantitative trait loci (QTLs) associated with partial Sclerotinia stem rot resistance in soybean plant introduction (PI) 391589B and Asgrow 2506.

The PI 391589B with partial Sclerotinia stem rot resistance was crossed with a susceptible cultivar IA 2053 to develop a mapping population of 94 F_2 derived lines. The population was evaluated for Sclerotinia stem rot resistance in the field in 2003 and 2004 and in the greenhouse in 2005. The population was also evaluated for genotypes with 109 polymorphic simple sequence repeat (SSR) markers. Both single marker analysis (SMA) and composite interval mapping (CIM) methods were used to determine the locations of the QTLs. The SMA revealed several markers from linkage group E significantly ($P<0.05$) associated with Sclerotinia stem rot resistance. With the CIM method, a QTL was identified near marker Satt185 on linkage group E in the 2005 greenhouse experiments. The QTL explained about 15.5% of the total phenotypic variance. The resistance allele of this QTL was from IA 2053. With the CIM method, another QTL was identified in the

2004 field experiment. The QTL is close to marker Satt212, which is also on linkage group E but is 11.5 cM away from marker Satt185. The QTL explained 53.1% of the total phenotypic variance. The resistance allele of this QTL was from PI 391589B.

The Asgrow 2506 with partial Sclerotinia stem rot resistance was crossed with another resistant cultivar NKS 19-90 to develop a mapping population of 140 F_4 derived lines. The population was evaluated for Sclerotinia stem rot resistance in the field in 2001 and 2002 and in the greenhouse in 2002 and 2003. The population was also evaluated for genotypes with 53 polymorphic SSR markers. The SMA revealed that marker Sat_327 on linkage group C1 was significantly ($P<0.05$) associated with Sclerotinia stem rot resistance. The CIM method also identified one QTL located near marker Sat_327 on linkage group C1, which explained about 12.2% of the total phenotypic variance. The resistance allele was from NKS 19-90. The QTLs identified can be used in maker assisted selection (MAS) in soybean breeding for Sclerotinia stem rot resistance.

Dedicated to my husband Guobin, my little girl Michelle Kang, and my expecting boy.

And also to the Guo family in Hebei – China.

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CHAPTER 1

INTRODUCTION

SOYBEAN

Soybean, *Glycine max* (L.) Merr., was domesticated in China from the wild annual soybean, *G. soja*, during the Chou Dynasty, from the 11th to the 7th century B.C. Soybean was introduced into North America by Samuel Bowen in the 1700's (Hymowitz and Harlan, 1983), and most of the U.S. soybean germplasm came from China, South Korea, and Japan (Morse et al., 1949). It was introduced for forage usage, but now soybean is mainly used for soybean meal and oil production in the U.S. Approximately 80% of edible fats and oils in the U.S. are derived from soybean.

Soybean is considered an oilseed crop and contributes over 50% of world oilseed production. Currently, soybean is grown in approximately 50 countries worldwide. The U.S. has been a leading soybean producer during the past half century with over 45% of world production prior to 2001 (FAO 2002). Today, the U.S., Brazil, China and Argentina are the four major soybean producers, accounting for more than 90% of the world production. In 2004, the U.S. production accounted for 40% of world production, while Brazil, Argentina, and China contributed 24%, 18%, and 8%, respectively (Soy Stats. 2005).

In the U.S., soybean is frequently rotated with corn (*Zea mays* L.) because the rotation can improve soil fertility and reduce diseases. The five major soybean production regions in the U.S. are the Western Corn Belt, Eastern Corn Belt, Southeast, Delta, and Atlantic states. In 2004, 30.4 million hectares were planted with soybean in the U.S. and the U.S. soybean production was 85.5 million metric tons (Soy Stats. 2005).

SCLEROTINIA STEM ROT

Sclerotinia stem rot, also called white mold, is one of the most serious soybean diseases in the U.S., especially in the north central region (Wrather et al., 1997). The disease was ranked second in the U.S. and sixth around the world among the most serious diseases reducing soybean yield (Wrather et al., 1997). In addition to yield loss, white mold has been reported to cause significant reduction in seed size, seed germination, and seed quality (Hoffman et al., 1998). White mold was first reported in the U.S. in 1924 (Grau and Hartman, 1999). In 1990 it became widespread in the Great Lakes States, and by 1992 it was prevalent in the North Central States.

White mold is caused by the fungal pathogen *Sclerotinia sclerotiorum* (Lib.) De Bary, a long-lived soil-borne fungus with a broad host range. It can be found in most regions where environments are cool and moist. *S. sclerotiorum* infects about 400 species and 220 genera in 60 plant families (Purdy, 1979). A number of plants such as soybean, dry beans, cabbage, canola, and sunflower are potential hosts. Non-host crops are corn, small grains, and all forage grasses (Boland and R.Hall, 1994).

Sclerotia, overwinter survival structure, produce apothecia in which ascospores start infection. Sclerotia are formed and matured from mycelium on the stem surface, and inside stems and pods. They fall to the soil surface and overwinter in the soil, and survival sclerotia germinate the following year or remain in the soil for a few years and still are viable (Grau, 1988).

Disease occurrence depends on germination of sclerotia, formation of apothecia, release of ascospores, a flowering host, and a favorable canopy microclimate. The canopy microclimate plays an important role in disease incidence. Canopy temperatures of <28°C and high relative humidity and soil moisture are needed for disease development (Boland and Hall, 1988). The disease cycle begins when apothecia form from sclerotia and ascospores are ejected from asci. The ascospores land on senescing flower petals to germinate (Sutton and Deverall, 1983), and hyphae colonize senescent tissues, then infection progresses into pod and node tissues. Cool and moist weather conditions during flowering favor *S. sclerotium* infection of soybean flower petals (Grau, 1988). Symptoms at the canopy level can be observed a few weeks after flowering. White fluffy mycelium often covers the lesions during high humidity periods and the lesioned part of the stem becomes bleached. The dark- colored sclerotia are formed inside and outside of the stems or pods.

Several practices have been recommended for the control of white mold on soybean (Grau et al., 1994). They include using resistant soybean varieties, widening rows, reducing plant populations, changing tillage practices, and using fungicides. Among these,

using resistant varieties is the most effective approach for the control of the disease (Kurle et al., 1998). Rotation with non-host crops such as corn or wheat can be beneficial because sclerotia germinate in non-host crop fields will reduce the infection load of a suitable host in the following year. Sclerotia germination is enhanced if the canopy moisture is increased by rapid and complete canopy closure, narrow row width, high population density, early planting, and high soil fertility (Grau and Radke, 1984). Integrating resistant varieties and adjusting cultural practices can minimize the effects of environmental factors.

Understanding the genetics of resistance to white mold is essential for the development of soybean varieties resistant to the disease. Resistance genes from known sources must be placed on a genetic linkage map before breeders can use molecular markers to speed the transfer of resistance genes into new high-yielding varieties.

SOURCES AND GENETICS OF RESISTANCE

No sources of complete soybean white mold resistance have been identified according to Kim and Diers (2000). Only partial resistance to *S. sclerotiorum* was found in some soybean genotypes in field tests (Boland and Hall, 1987; Grau et al., 1982; Kim et al., 1999; Nelson et al., 1991). The cultivar NKS19-90 is currently the major source for the development of resistant soybean varieties. Identification of better sources of resistance to white mold in soybean plant introductions (PI) has received major attention in the past few years in the North Central Soybean Research Program – White Mold Coalition. Over 5,500 PIs have been tested for resistance to white mold, but only a few PIs were found more

resistant than NKS19-90 (Hoffman et al., 2002). Of the five resistant PIs brought to Michigan State University in 2001, PI 391589B appeared to be best adapted to Michigan growing conditions. PI 391589B has been successfully crossed with IA 2053 which is susceptible to white mold, but are desirable for other agronomic traits.

Kim and Diers (2000) pointed out that the inheritance of partial resistance to white mold was under multiple loci control with a broad sense heritability estimate of 0.59. They studied the inheritance of partial resistance to white mold in NKS19-90 and identified three QTLs conferring this resistance. Two QTLs from linkage groups C2 and M were correlated with an escape mechanism. The resistance allele on linkage group C2 was significantly associated with shorter plant height and a smaller lodging index. The resistance allele on linkage group M was significantly associated with early flowering. However, the third QTL from linkage group K might be related to a physiological mechanism. The three QTLs from linkage group C2, M and K explained 7.8%, 9.2%, and 9.6% of variation in the disease severity index, respectively.

Arahana et al. (2001) identified 28 putative QTLs using single marker analysis (SMA) for white mold resistance on 15 different linkage groups from five populations, in which the resistance sources were Corsoy79, Dassel, DSR173, Vinton81, and NKS 19-90, and found that individual QTLs explained the disease variation range from 4 to 10%. Seven QTLs on seven different linkage groups (A2, D1a, D1b, F, G, L, and O) were identified in more than one population. The five populations shared the same white mold susceptible parent Williams 82, in which the authors identified a few additional QTLs on different

linkage groups. The seven QTLs were in different linkage groups from those identified by Kim and Diers (2000).

MECHANISMS OF SOYBEAN RESISTANCE

The soybean white mold resistance mechanisms include disease escape and physiological resistance. Most factors which affect the canopy environment are escape mechanisms, such as plant architecture, lodging, and maturity (Kim and Diers, 2000). However, there are some physiological mechanisms which result in disease resistance, including glyceolin, a phytoalexin, which is induced when soybean is infected with ascospores or mycelium (Sutton and Deverall, 1984). White mold secretes oxalic acid, and cell-wall-degrading enzymes such as polygalacturonase, cellulase, and xylanase, which are required for *S. sclerotiorum* infection (Cessna et al., 2000; Marciano et al., 1983). Godoy et al. (1990) used mutants to demonstrate that oxalic acid is a pathogenecity determinant. Oxalic acid also lowers extracellular pH, allowing optimum activity of cell-wall-degrading enzymes (Marciano et al., 1983).

Donaldson et al. (2001) transformed the oxalate oxidase *gf-2.8* (germin) gene from wheat and found that the transgenic soybean showed greatly reduced disease progress. This enzyme oxidizes oxalic acid to carbon dioxide and hydrogen peroxide. Partially resistant soybean genotypes may be tolerant to or metabolize oxalic acid and polygalacturonase.

SOYBEAN GENETIC LINKAGE MAP

The cultivated soybean is considered a diploidized allotetraploid (Shoemaker et al., 1996) with chromosome number 2n=2x=40. The first 20 consensus linkage groups (Cregan et al. 1999a; Cregan et al. 1999b) developed from over 1300 markers including 606 SSR markers were assumed to represent the 20 pairs of soybean chromosomes. The 20 linkage groups were named A1, A2, B1, B2, C1, C2, D1a, D1b, D2, E, F, G, H, I, J, K, L, M, N, and O. Song et al. (2004) added another 391 SSR markers to the integrated linkage map.

The current soybean genetic linkage map contains over 2,000 molecular markers, of which about 1,000 are simple sequence repeat (SSR) DNA markers. The soybean composite map (Song et al., 2004) can be used to help identify where the white mold resistance genes are located in the genome. In our study all the SSR markers from the 20 linkage groups were tested for polymorphism and all polymorphic markers were used for identification of QTL conferring white mold resistance.

MARKER ASSISTED SELECTION

Pathogens easily overcome plant resistance that is due to a single gene and produce a new pathogenic strain. Pyramiding of multiple resistance genes can enhance the resistance and also make the resistance more durable. Marker assisted selection (MAS) will assist breeding by identifying individuals with different resistance alleles at differrent loci, allowing development of new varieties with multiple resistance genes. It provides consistent results that do not rely on a pest-plant interaction under certain environmental conditions.

By identifying QTLs associated with desirable traits, DNA markers closely associated with the QTLs will be available to help identify progenies with genes of interest. MAS makes selection more efficient by testing for the presence or absence of certain alleles of markers linked with QTLs associated with desirable traits and providing faster and more reliable identification of valuable plants. MAS can be helpful under the following conditions: 1) if space available to select phenotypical traits in the breeding nursery is limited; 2) if no disease pressure is present during the breeding process; 3) if only one or two years phenotypic data are available; and 5) if breeders have to wait for several generations for the selection in traditional breeding. For MAS, only a small piece of tissue is needed to isolate DNA and use selected markers to test if the plant carries genes of interest.

Graef et al. (National Sclerotinia Initiative abstract, 2005) developed F4-derived soybean lines homozygous for the desired marker alleles for the 8 QTL on 7 different linkage groups (Arahana et al., 2001). However, no significant resistance improved lines were identified until now.

OBJECTIVES

The partially resistant soybean variety NKS 19-90 was the major resistance source for the development of resistant soybean varieties. Variety Asgrow 2506 also showed partial resistance and a population was developed by crossing NKS 19-90 and Asgrow 2506. A new white mold resistance source, PI 391589B, appeared to be the best adapted to

Michigan growing conditions. A few populations were developed from PI 391589B.

The objectives of my research were:

1) Locate the genes conferring white mold resistance in the new resistance source PI 391589B in the current soybean genetic linkage map.

2) Determine whether Asgrow 2506 contains genes conferring partial white mold resistance.

3) If genes conferring partial white mold resistance were identified determine if the white mold resistance can be increased by combining resistance genes from Asgrow 2506 and NKS 19-90.

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CHAPTER 2

GENETIC MAPPING OF QTLS UNDERLYING PARTIAL SCLEROTINIA STEM ROT RESISTANCE IN SOYBEAN PI 391589B

ABSTRACT

Sclerotinia stem rot is an important yield reducing disease in soybean in the Midwestern states of the U.S.. Soybean plant introduction (PI) 391589B has partial resistance to the disease. The objective of this study was to identify quantitative trait loci (QTLs) associated with partial Sclerotinia stem rot resistance in PI 391589B. PI 391589B was crossed with susceptible cultivar IA 2053 to develop a mapping population of 94 F_2 derived lines. The population was evaluated for Sclerotinia stem rot resistance in the field in 2003 and 2004 and in the greenhouse in 2005. The population was also evaluated for genotypes with 109 polymorphic simple sequence repeat (SSR) markers. Single marker analysis (SMA) revealed several markers from linkage group E significantly ($P<0.05$) associated with soybean Sclerotinia stem rot resistance. Composite interval mapping (CIM) method identified a QTL in the 2005 greenhouse experiments located near marker Satt185 on the lower part of linkage group E, which explained about 15.5% of the total phenotypic variance in greenhouse experiment. The resistance allele near the marker Satt185 was from

IA 2053. The second QTL located near marker Satt651 on the upper part of linkage group E was identified in the 2004 field experiment and explained 53.1% of the total phenotypic variance. The resistance allele near marker Satt651 was from PI 391589B.

INTRODUCTION

Sclerotinia stem rot, also called white mold, is caused by the fungal pathogen *Sclerotinia sclerotiorum* (Lib.) De Bary. *S. sclerotiorum* is a soil borne fungus which infects a large number of crops including soybean. The severity of Sclerotinia stem rot is related to environmental conditions, with cool temperatures (below 25°C) and moist canopy conditions favoring *S. sclerotiorum* growth (Grau, 1988). The infection process begins by colonization of the soybean flower petals by *S. sclerotiorum* ascospores. Infection then spreads to pods, nodes, and stems, and finally may result in premature plant death (Grau, 1988). Sclerotinia stem rot has been prevalent in the north central United States since 1992 (Wrather et al., 1997), causing yield loss, and reduction in seed size and quality (Hoffman et al., 1998).

The most effective approach to control Sclerotinia stem rot is to use resistant varieties (Kurle et al., 1998). No sources of complete resistance to the disease has been identified, and only sources of partial resistance, which show less disease severity with less yield loss and better seed quality compared with susceptible ones, have been identified (Kurle et al., 2001). Partial Sclerotinia stem rot resistance in soybean is a quantitative trait (Kim and Diers, 2000). Kim and Diers (2000) identified three quantitative trait loci (QTLs)

associated with Sclerotinia stem rot resistance in the soybean variety NKS 19-90. Arahana et al. (2001) identified twenty-eight putative QTLs from five recombinant inbred line (RIL) populations. However, correlations of these putative QTLs with Sclerotinia stem rot resistance have not yet been confirmed in other populations.

QTLs conferring disease resistance could vary among different sources of resistance. Identification of QTLs from other resistant sources could help understand relationship among resistance genes and evolution of resistance. DNA markers closely linked to QTLs could be used in marker assisted selection in soybean breeding for Sclerotinia stem rot resistance, which would accelerate the breeding process and facilitate combining different resistance genes into one variety. The objective of the study was to identify QTL conferring partial Sclerotinia stem rot resistance in soybean PI 391589B.

MATERIALS AND METHODS

Mapping population

A mapping population of 94 F_2 derived lines was developed from a cross of soybean PI 391589B x IA 2053. PI 391589B was a germplasm collected from China (USDA-ARS), National Genetic Resources Program) with partial Sclerotinia stem rot resistance (Hoffman et al., 2002). IA 2053 is a food-grade variety developed by Dr. Walter Fehr at Iowa State University and is susceptible to Sclerotinia stem rot. F_2 plants were individually harvested to create $F_{2:3}$ lines. Each $F_{2:3}$ line was advanced to the $F_{2:4}$ generation by harvesting a single seed from each plant and bulking the single seed of all plants of the $F_{2:3}$ line. $F_{2:5}$ lines were

produced in the same way.

Population genotyping

Genotyping were carried out at the F_2 generation of the population. Unopened trifoliolate leaves were collected from each F_2 plant in the field and kept on ice before the samples were transported to the laboratory. The leaf samples were kept at -80°C for at least 48 hours and then lyophilized for approximately 72 hours. DNA was extracted from the dried leaf tissues using a modified CTAB method (Kisha et al., 1997).

Simple sequence repeat (SSR) markers were used to genotype the population. The SSR primer sequences were obtained according to Song et al. (2004). SSR primers were synthesized by the Genomics Technology Support Facility at Michigan State University. A total of 1132 SSR markers were screened for polymorphism between the two parents. The bulked F_2 population DNA was added to confirm polymorphism in the population. A total of 109 polymorphic SSR markers were selected to genotype the entire population.

DNA amplification of SSR markers was performed using a 15 µl polymerase chain reaction (PCR) mix consisting of 1.0x PCR buffer (10 mM Tris-HCl, 5 0 mM KCl, 0.01% Gelatin, pH = 8.3), 3.0 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP, and dTTP (Sigma-Aldrich, St. Louis, MO), 0.3 µM each of forward and reverse primers, 100 ng of genomic DNA, and 1 unit of *Thermus aquaticus (Taq)* DNA polymerase. The amplification consisted of an initial denaturation of 4 min. at 94 °C followed by 43 cycles of 25 sec. denaturation at 94 °C, 25 sec. annealing at 47 °C, and 25 sec. extension at 68 °C, and finally an additional 7 min. extension at 72 °C before cooling down to 4 °C in a MJ Tetrad™

thermal cycler (MJ Research, Waltham, MA). The PCR products were analyzed in a 6% non-denaturing polyacrylamide gel system as described by Wang *et al.* (2003).

Population phenotyping

The $F_{2:3}$ and $F_{2:4}$ populations were tested for Sclerotinia stem rot resistance by field inoculation in 2003 and 2004. Field experiments were performed at the Agronomy Farm of the Department of Crop and Soil Sciences at Michigan State University (East Lansing, Michigan). A randomized complete block design (RCBD) was used with three replications of the parental genotypes and 94 $F_{2:3}$ lines in 2003 and six replications of the parental genotypes and 94 $F_{2:4}$ lines in 2004. Each plot had one 3 m long row of approximately 30 plants; rows were spaced 38 cm apart. After each inoculation the fields were sprinkler-irrigated with approximately 2.5 mm of water every evening for three weeks.

The $F_{2:3}$ population was planted on May 28th 2003 and was tested for Sclerotinia stem rot resistance by the cut-petiole inoculation based on the method of of Del Rio et al. (2001) using *S. sclerotiorum* soybean isolate HT 105 (Provided by Dr. Glen Hartman, University of Illinois). The HT 105 isolate was maintained by subculturing every two weeks onto potato dextrose agar (PDA) medium. To prepare inocula for field experiments, a plug of mycelia was transfered to PDA medium in 120 mm x 30 mm Petri dishes maintained on the lab bench at room temperature (22-25°C) until mycelia reached the edge of the plate in approximately three days. A 1 cm diameter, 8 mm deep plug of mycelium was removed using an inverted 1 ml pipette filter tip (USA Scientific, Ocala, FL) and was used to inoculate a soybean plant in the field. The mycelium plugs loaded in 1 ml pipette tips were

prepared in the early morning of the inoculation day and kept on ice until placed on the plants. The second youngest fully opened trifoliolate leaf was removed with a razor blade approximately 5 cm above the point of attachment to the petiole. The cut petiole was forced into the wide end of the pipette tip containing the mycelial plug, to assure contact of the cut petiole surface with the mycelia. Each replication was inoculated on a separate day, August 13th, August 22nd and August 29th, respectively. Plants were observed daily after inoculation until the first plant showed apical wilting symptoms. Plants were subsequently evaluated for wilting twice a week for the first 7 weeks after each inoculation. The wilted apical parts were removed after data collection to prevent plant death to allow for $F_{2:4}$ seed production. AUDPC (Area Under Disease Progress Curve) was calculated for each line according to the formula of Shaner and Finney (Shaner and Finney, 1977).

The $F_{2:4}$ population was planted on June 8th 2004. In order to expedite field inoculations a new method (tip drop method) was developed (Chen and Wang, 2005). *S. sclerotiorum* HT 105 mycelia were cultured in PDA medium for three days at room temperature (22-25°C). Twenty, 8 mm diameter plugs of *S. sclerotiorum* HT 105 mycelia were put into 2 L of autoclaved liquid potato dextrose broth (PDB), and cultured for five days at room temperature with shaking at 100 RPM. The mycelial culture was blended for 10 seconds at high speed using a 1350 ml household blender (BlenderMaster, Hamiton Beach/Proctor-Silex Inc, Mexico) to create a uniform suspension and was then transferred to 500 ml wash bottles. Approximately 3 ml of the mycelial suspension (sufficient to allow runoff) was applied to the apical meristems and the youngest one or two axillary meristems

of each plant. Inoculation of the six replicate blocks were made on different days, August 10th, August 13th, August 17th, August 19th, August 23rd, and August 24th to avoid the rain wash off or environmental effect. When the majority of the plants reached physiological maturity (R7) (Fehr and Caviness, 1977) all inoculated plants in each row were individually rated for disease severity based on the rating system of Grau *et al.* (1982), where 0 = no symptom, 1 = lesions on lateral branches only, 2 = lesions on the main stem but little or no effects on pod fill, and 3 = lesions on main stem resulting in poor pod fill or plant death. A disease severity index (DSI) was calculated for each line using the formula DSI=100 x (Σ r/3n) (r = rating of each plant; n = number of plants rated).

Plant height and lodging scores were collected before harvest in 2003 and 2004 field. Plant height was determined as the average length of plants in a plot from the ground to the uppermost node of the plant at maturity. Lodging notes were recorded on a scale of 1 to 5 according to the following criteria: 1.0 - almost all plants erect; 2.0 - either all plants leaning slightly, or a few plants down; 3.0 - either all plants leaning moderately (45°), or 25 to 50% down; 4.0 - either all plants leaning considerably, or 50 to 80% down; 5.0 - all plants down.

The $F_{2:5}$ population was tested by greenhouse inoculation in 2005 using a randomized complete block design with three replications. Each replication consisted of two pots with approximately ten plants. To facilitate infection, an inoculation chamber was made by covering a 5 m X 1.5 m X 1.5 m section of bench with plastic. Two misters were placed at each end of the plastic chamber. The plants were grown in the greenhouse outside the

plastic chamber for about three weeks (between V2 and V3 stages) and then transferred to the inoculation chamber. Inoculation was done using the tip drop method previously described. Approximately 0.5 ml of the mycelial suspension was applied to the apical meristems. After each inoculation the plastic chamber was covered and the misters emitted water mist for one minute every five minutes. The growth chamber held one replication of the parental genotypes and 94 $F_{2:5}$ lines. Three replications were sequentially done in March and April of 2005. The plants were scored for mortality on day 5, 8 and 12 after inoculation. AUDPC (Area Under Disease Progress Curve) was calculated for each line according to the formula of Shaner and Finney (1977).

The computer program JoinMap 3.0 (Van Ooijen and Voorrips, 2001) and the Kosambi mapping function (Kosambi, 1944) were used to determine the linkage relationships among the polymorphic SSR markers. The LOD groupings threshold was set to 3.0 to divide markers into 23 linkage groups. The best position of each marker was searched by comparing the goodness-of-fit for each tested position to determine the order and distance among markers within each linkage group. The linkage map resulted from JoinMap analysis was used as map input in the QTL analysis using WinQTLcart V2.5 (Wang et al., 2005).

The simple linear regression model from WinQTLcart V2.5 was used to identify markers significantly (P value=5%) associated with Sclerotinia stem rot resistance in soybean. The LOD threshold used to declare the putative QTLs associated with Sclerotinia stem rot resistance in CIM method was 2.5. The estimated QTL position was under the

peak of LOD score curve.

RESULTS

Distribution of Phenotypic Data

The AUDPC values of the mapping population obtained in the 2003 field evaluation were normally distributed with a range from 5 to 30 (Figure 2-1). The DSI of the population from the 2004 field test was approximately normally distributed with a range from 0 to 40 (Figure 2-1). For both field evaluations, the resistant and susceptible parents fell near the extremes of the distribution. The AUDPC values of the population in the 2005 greenhouse evaluation was also approximately normally distributed with a range from 1 to 8 (Figure 2-1). The AUDPC values obtained from the greenhouse evaluation was smaller than those from the field evaluation because younger plants were inoculated and higher humidity was maintained in the greenhouse relative to the field. Most susceptible plants died within 12 days after inoculation in the greenhouse evaluation versus 7 weeks after inoculation in the field evaluation. In the 2004 field evaluation and the 2005 greenhouse evaluation, the tip drop inoculation method was used. However, the AUDPC disease rating system was used in the greenhouse evaluation while DSI was used in the field evaluation due to slower disease development and limited time available to score disease in the field.

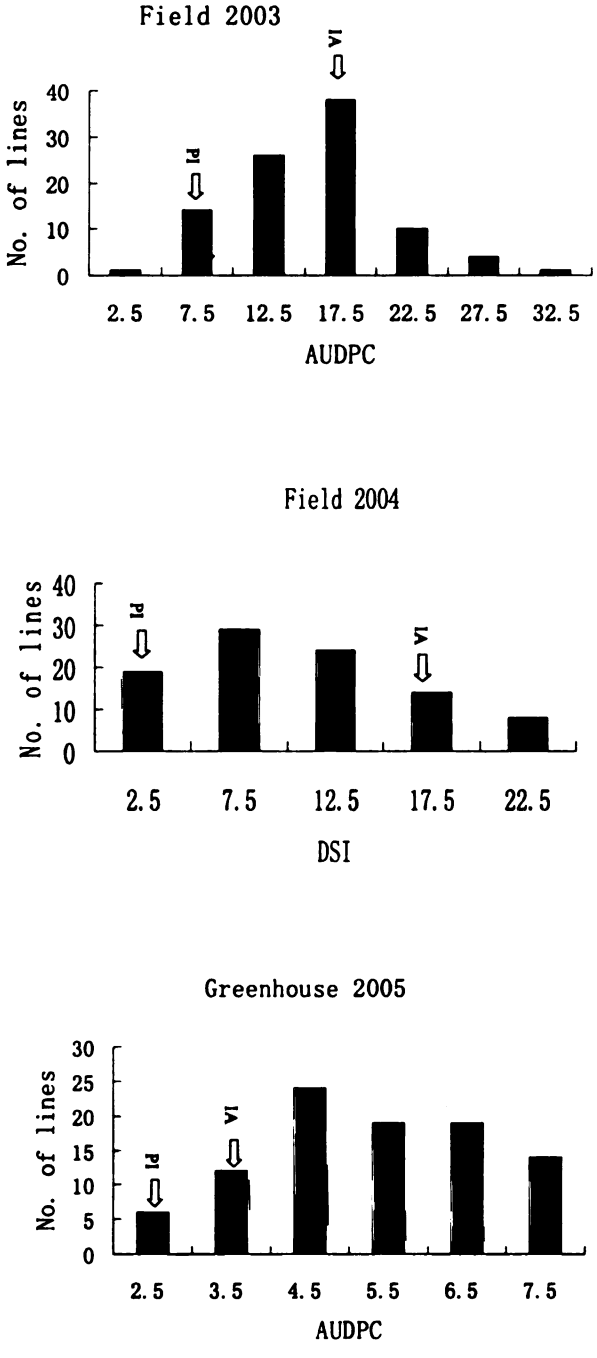

Figure 2-1. Distributions of the AUDPC values in the 2003 field evaluation and the 2005 greenhouse evaluation and the DSI in the 2004 field evaluation of the 94 F_2–derived lines from the cross PI 391589B x IA 2053.

Identification of QTL for Sclerotinia stem rot resistance

Of the 1132 SSR markers tested for polymorphism between the two parents, 109 were polymorphic. The 109 polymorphic markers were placed into 23 linkage groups with a LOD threshold of 3.0 using Joinmap 3.0. The 23 linkage groups were segments of 18 linkage groups on the integrated soybean linkage map (Song et al., 2004). Fifteen markers were placed on linkage group E (Figure 2-2). The total map distance of the 23 linkage groups with 88 SSR markers was 673.6 cM with average interval length 7.7 cM. The total map distance was 399.9 cM using the map of Song et al. (2004) and represented 16% of the soybean genome coverage.

Both single marker analysis (SMA) and composite interval mapping (CIM) methods were used in the QTL analysis. Single marker analysis (Table 2-1) revealed significant markers on linkage groups F, I, and E significantly ($P<0.05$) associated with soybean Sclerotinia stem rot resistance. All the resistance alleles of the significant markers identified with the 2003 and 2004 field data were from PI 391589B. However, the resistance alleles of the significant markers identified with the 2005 greenhouse data were from IA 2053. The CIM method identified one QTLwith the 2003 field data with a LOD score of 2.6 (Figure 2-2A). The QTL was located near marker Satt483 on linkage group E and explained 12.2% of the total phenotypic variance. Another QTL was identified with the 2004 field data with a LOD score of 15.1(Figure 2-2B). The QTL was located near marker Satt651 on linkage group E and explained 53.1% of the total phenotypic variance. The third QTL was identified with the 2005 greenhouse data with a LOD score of 3.7 (Figure

2-2A). This QTL was located near marker Satt185 on linkage group E and explained 15.5% of the total phenotypic variance.

The correlations between DSI and plant height were not significant at 0.05 probability level in 2003 and 2004 field. The correlations between DSI and lodging scores in 2003 and 2004 were 0.33 ($p<0.004$) and 0.30 ($p<0.003$), respectively. However, no significant association was identified between Sclerotinia stem rot resistance and lodging in SMA and QTL analysis.

Table 2-1. Markers significantly associated with Sclerotinia stem rot resistance in single marker analysis. Markers significant at the 5%, 1%, 0.1% and 0.01% levels were indicated by *, **, ***, and **** respectively. Linkage group names and relative position for the markers were assigned according to the Soybean Composite Map (Song et al., 2004).

Marker	Linkage Group	Position†	P-value			Resistance Source‡
			2003 AUDPC	2004 DSI	2005 AUDPC	
Satt512	E	?	0.064	0.835	0.688	NRS
BR347343	E	?	0.288	0.9	0.405	NRS
Satt411	E	12.92	0.014 *	0.011 *	0.277	PI 391589B
Satt384	E	19.3	0.11	0.066	0.23	NRS
Satt720	E	20.8	0.095	0.019 *	0.314	PI 391589B
Satt651	E	32.1	0.005 **	0.000 ****	0.931	PI 391589B
Satt212	E	32.27	0.019 *	0.006 **	0.769	PI 391589B
Satt606	E	39.77	0.276	0.594	0.022 *	IA 2053
Satt699	E	41.24	0.239	0.432	0.001 ***	IA 2053
Satt706	E	43.36	0.243	0.381	0.002 **	IA 2053
Satt491	E	43.64	0.305	0.693	0.003 **	IA 2053
Satt185	E	44.76	0.066	0.262	0.000 ***	IA 2053
Satt483	E	44.98	0.894	0.185	0.011 *	IA 2053
Satt263	E	45.4	0.333	0.838	0.001 ***	IA 2053
Satt685	E	56.7	0.198	0.948	0.584	NRS
AW186493	F	?	0.034 *	0.015 *	0.147	PI 391589B
Satt149	F	18.13	0.025 *	0.035 *	0.859	PI 391589B
Satt419	I	21.9	0.037 *	0.035 *	0.477	PI 391589B

† "?" means no position available on the soybean composite map.
‡ NRS = no resistance source available at 0.05 probability level.

Figure 2-2A

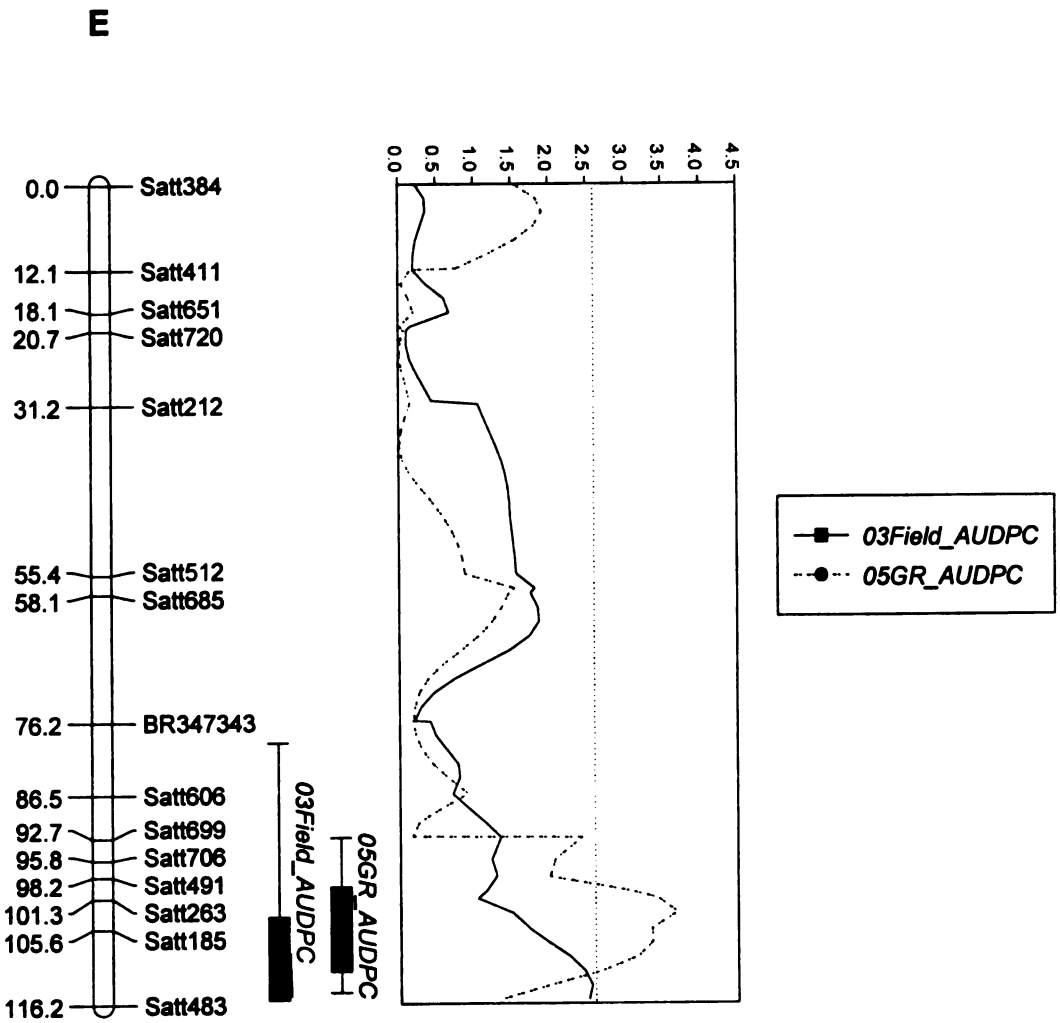
E
0.0
Satt384
12.1
Satt411
18.1
Satt651
20.7
Satt720
31.2
Satt212
55.4
Satt512
58.1
Satt685
76.2
BR347343
86.5
Satt606
92.7
Satt699
95.8
Satt706
98.2
Satt491
101.3
Satt263
105.6
Satt185
116.2
Satt483
03Field_AUDPC
05GR_AUDPC
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
03Field_AUDPC
05GR_AUDPC

Figure 2-2B

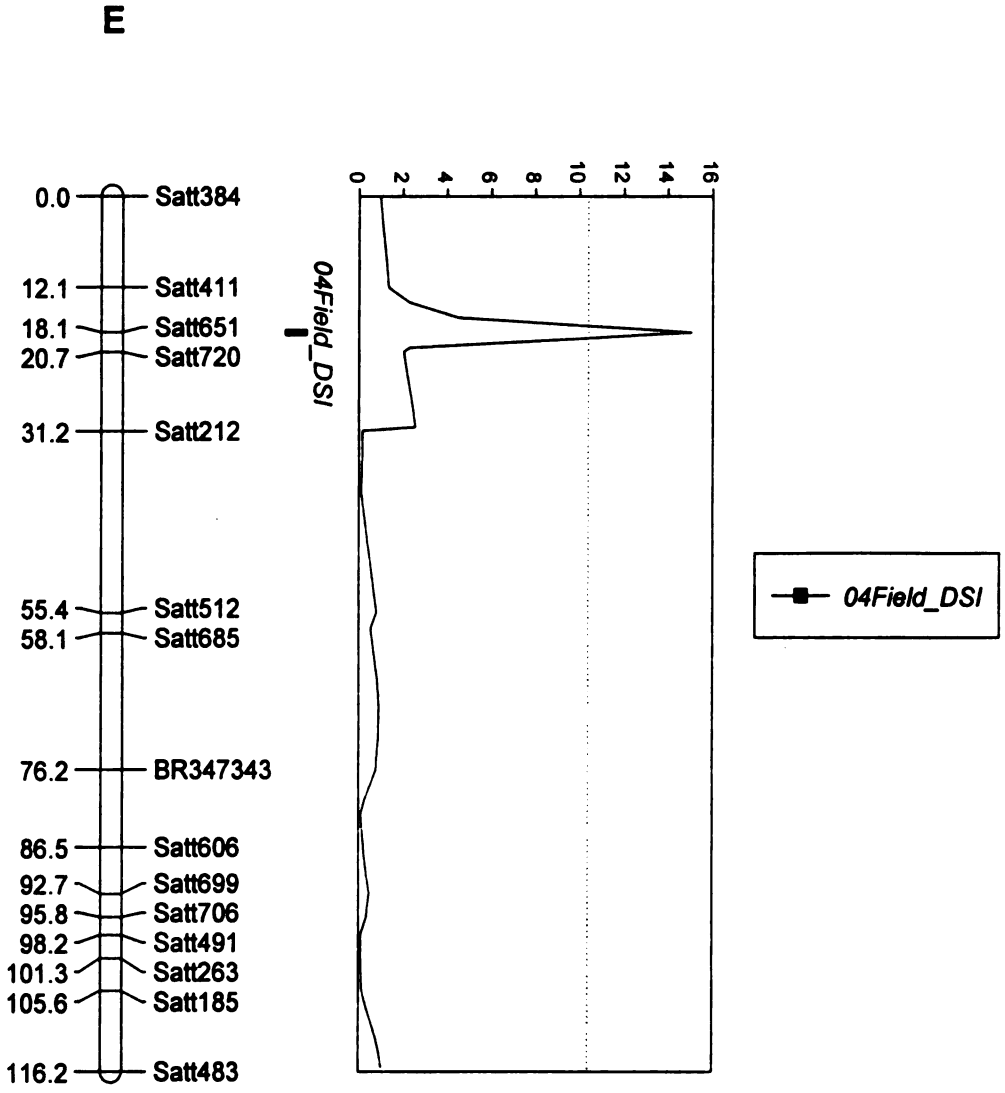

Figure 2-2. Putative QTLs associated with Sclerotinia stem rot resistance on linkage group E from 2003 and 2004 field and 2005 greenhouse experiments. The map distances between the markers are given in cM (centiMorgans). The linkage groups are named according to Song et al. (2004). One QTL was identified around SSR marker Satt263 and Satt185 on linkage group E in 2003 field and 2005 greenhouse experiments (A) and a second QTL was identified around SSR marker Satt651 on linkage group E in 2004 field experiment (B). The LOD threshold after 1000 permutation was 2.6 in 2003 field and 2005 greenhouse but it was 10.4 in 2004 field data.

DISCUSSION

The results obtained in this study indicate two putative QTLs on linkage group E contributed to soybean Sclerotinia stem rot resistance. The resistance allele of one putative QTL near marker Satt651 on linkage group E was from PI 391589B. This QTL was identified by SMA in both 2003 and 2004 field experiments and also by CIM in the 2004 field experiment. The resistance allele of the other putative QTL near marker Satt185 on linkage group E was from IA 2053. This QTL was identified by both SMA and CIM in the 2005 greenhouse experiment and also suggested by the CIM in the 2003 field experiment. The QTLs identified in the field and greenhouse environments belong to two different regions of linkage group E. The QTL with resistance allele from PI 391589B was observed under field conditions, and the QTL with resistance allele from IA 2053 was observed primarily under greenhouse conditions.

Previous studies by Arahana et al. (2001) identified one marker OP_m12 on linkage group E significantly associated with soybean Sclerotinia stem rot resistance. The putative QTL close to marker Satt651 on linkage group E was approximately 10 cM from their significant marker Op_m12, based on the integrated soybean linkage map (Song et al., 2004). The resistance allele at the marker OP_m12 identified by Arahana et al. (2001) was from a susceptible variety, Williams 82, while the resistance allele we identified was from a partially resistant germplasm, PI 391589B. Since these two markers are only 10 cM away from each other on the same linkage group, the putative QTLs might be the same locus carrying the same or different resistance alleles.

The QTL located near marker Satt185 on linkage group E has not been identified in any other studies and the resistance allele was from the susceptible parent, IA 2053. This QTL was identified in both the 2003 field evaluation and the 2005 greenhouse evaluation, suggesting the QTL may confer resistance consistently expressed across different environments.

The cut-petiole inoculation method used in the 2003 field experiment allowed easier pathogen infection due to the lack of an epidermal barrier from the host plant. Tip drop inoculation used in the 2004 field and 2005 greenhouse experiments is more similar to natural infection because the pathogen was allowed to infect the plants without wounding the plants. Different disease rating systems were used for the tip drop method in the greenhouse versus the field because of differences in the speed of disease development and in labor intensity in disease rating. The differences in the speed of disease development in the greenhouse and in the field were likely due to the differences in the environmental conditions during disease development.

The Sclerotinia stem rot resistance QTL near Satt651 from PI 391589B was identified in two years' field evaluations. The other Sclerotinia stem rot resistance QTL near Satt185 from IA 2053 was identified in one year's field evaluation and the greenhouse evaluation. None of the resistance QTL was identified in all evaluations, indicating the expression of the resistance was affected by the environment.

It is important to have a sufficient number of polymorphic markers and reproducible phenotypic data to identify useful QTLs. The 109 polymorphic markers used in the study

were not enough to cover the twenty linkage groups of soybean. However, the new markers identified on linkage group E will provide information for future breeding for soybean Sclerotinia stem rot resistance. Breeding process will be accelerated if breeders test the markers identified in this study to be associated with soybean Sclerotinia stem rot resistance to start the primary screening.

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CHAPTER 3

GENETIC MAPPING OF QTLS UNDERLYING PARTIAL SCLEROTINIA STEM ROT RESISTANCE IN SOYBEAN VARIETY ASGROW 2506

ABSTRACT

Sclerotinia stem rot is an important yield reducing disease in soybean in the Midwestern states of the U.S..The objective of this study was to identify quantitative trait loci (QTLs) associated with partial Sclerotinia stem rot resistance in soybean variety Asgrow 2506. The Asgrow 2506 with partial Sclerotinia stem rot resistance was crossed with another partially resistant variety NKS 19-90 to develop a mapping population of 140 F_4 derived lines. The population was evaluated for Sclerotinia stem rot resistance in the field in 1998, 1999, and 2002, and also in the greenhouse in 2002 and 2003. The population was tested with 53 polymorphic simple sequence repeat (SSR) markers. Single marker analysis (SMA) revealed marker Sat_327 from linkage group C1 significantly ($P<0.05$) associated with soybean Sclerotinia stem rot resistance. Composite interval mapping (CIM) method also identified a QTL located near marker Sat_327. The QTL explained about 12.2% of the total phenotypic variance in 2003 greenhouse experiment. The resistance allele near the marker Sat_327 was from NKS 19-90.

INTRODUCTION

Sclerotinia stem rot (white mold) of soybean is caused by the fungal pathogen *Sclerotinia sclerotiorum* (Lib.) De Bary. *S. sclerotiorum* can be found in most areas where environments are cool and moist with a host range of about 400 species, 220 genera, and 60 plant families (Purdy, 1979). The infection process begins by colonization of the soybean flower petals by *S. sclerotiorum* ascospores. Infection then spreads to pods, nodes, and stems, and finally may result in premature plant death (Grau, 1988). Sclerotinia stem rot has been prevalent in the north central United States since 1992 (Wrather et al., 1997), causing yield loss and seed size and quality reduction (Hoffman et al., 1998).

Several practices have been recommended for the control of white mold on soybean (Grau et al., 1994). The recommended practices include using resistant soybean varieties, widening rows, reducing plant populations, changing tillage practices, and using fungicides. Among these, using resistant varieties is the most effective approach to control the disease (Kurle et al., 1998).

Although no sources of complete resistance to the disease are available, sources of partial resistance have been identified (Kim et al. 1999; Hoffman et al. 2002). Kim et al. (1999) evaluated 18 soybean genotypes for Sclerotinia stem rot resistance in six Michigan environments over 3 years and found that NKS 19-90, Asgrow 2506, Colfax, and Corsoy 79 had the greatest resistance. Hoffman et al. (2002) evaluated 6,520 soybean plant introductions (PIs) for Sclerotinia stem rot resistance and found 68 PIs with partial resistance to the disease.

Partial Sclerotinia stem rot resistance in soybean is a quantitative trait (Kim and Diers, 2000). Kim and Diers (2000) studied the inheritance of partial resistance to white mold in NKS 19-90 and identified three QTLs conferring partial resistance to the disease in NKS 19-90. Arahana et al. (2001) identified twenty-eight putative QTLs from five recombinant inbred line (RIL) populations. However, correlation between these putative QTLs and Sclerotinia stem rot resistance has not yet been confirmed. QTLs conferring disease resistance could vary among different sources of resistance. Identification of QTLs from other resistant sources could help understand genetic variation associated with disease resistance. The objective of this study was to identify QTLs conferring partial Sclerotinia stem rot resistance in soybean variety Asgrow 2506 and to determine if the resistance to white mold can be increased by combining resistance genes from Asgrow 2506 and NKS 19-90.

MATERIALS AND METHODS

Mapping population

The mapping population consisted of 140 F_4-derived lines developed from a cross of NKS 19-90 and Asgrow 2506 at Michigan State University by Dr. Brian Diers. NKS 19-90 was developed by Northrup King Company with a high level of partial Sclerotinia stem rot resistance. Asgrow 2506 was developed by Asgrow Seed Company with partial Sclerotinia stem rot resistance. Single-seed descent was used to advance the population to the F_4 generation. F_4 -derived lines were tested for partial Sclerotinia stem rot resistance.

Population genotyping

Unopened trifoliolate leaves from two weeks old plants were collected from 10 plants of each F_4 derived line in the greenhouse and kept on ice until transported to the laboratory. The leaf samples were kept at -80°C for at least 48 hours and then lyophilized for approximately 72 hours. DNA was extracted from the dried leaf tissues using a modified CTAB method (Kisha et al., 1997).

Simple sequence repeat (SSR) markers were used to genotype the population. The SSR primer sequences were obtained according to Song et al. (2004). SSR primers were synthesized by the Genomics Technology Support Facility at Michigan State University. A total of 1132 SSR markers were screened for polymorphism between the two parents. The bulked F_4 population DNA was also used to confirm polymorphism in the population. Fifty three polymorphic SSR markers were selected to genotype the entire population.

DNA amplification of SSR markers was performed using a 15 µl polymerase chain reaction (PCR) mix consisting of 1.0x PCR buffer (10 mM Tris-HCl, 50 mM KCl, 0.01% Gelatin, pH = 8.3), 3.0 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP, and dTTP (Sigma-Aldrich, St.Louis, MO), 0.3 µM each of forward and reverse primers, 100 ng of genomic DNA, and 1 unit of *Thermus aquaticus (Taq)* DNA polymerase. The amplification consisted of an initial denaturation of 4 min at 94 °C followed by 43 cycles of 25 sec denaturation at 94 °C, 25 sec. annealing at 47 °C, and 25 sec extension at 68 °C, and finally an additional 7 min extension at 72 °C before cooling down to 4 °C in a MJ Tetrad™ thermal cycler (MJ Research, Waltham, MA). The PCR products were analyzed in a 6%

non-denaturing polyacrylamide gel system as described by Wang et al. (2003).

Population phenotyping

In 1998 and 1999 field phenotypical data in Michigan and Illinois were collected by Dr. Brian Dier's group. The F_4-derived lines and parents were evaluated on the Agronomy Farm at Michigan State University in East lansing, Michigan and at the Crop Sciences Research and Education Center in Urbana, Illinois. Both enviroments were planted with a randomized complete block design (RCBD) with two replications. The plots in East Lansing were three, 6 m rows spaced 38 cm apart and the plots in Urbana were six, 3.7 m rows spaced 19 cm apart. The plots were inoculated with sclerotia collected from harvested common bean (*Phaseolus vulgaris* L.) by evenly spreading over the field from one week prior to the earlist soybean line having 50% of its plants reaching the R1 growth stage (Fehr and Caviness, 1977).

In 2002 the population was evaluated for white mold resistance on the Agronomy Farm at Michigan State University in East Lansing, Michigan. The field was artificially inoculated with sclerotia of *S. sclerotiorum* using the same method from last description. Both parents and the population of F_4-derived lines were planted in a RCBD with 2 replications. The lines were planted in 6-row plots with 38 cm row spacing and a length of 4.2 m. The fields were sprinkler-irrigated (approximately 2.5 mm) every evening from approximately one week prior to the first line onset of blooming (R1) (Fehr and Caviness, 1977) until completion of flowering of all lines.

The plots were rated for disease severity based on the rating system of Grau et al.

(1982) at approximately the beginning of physiological maturity (R7) (Fehr and Caviness, 1977). Thirty plants in the center rows of plots were individually rated on a scale of 0 to 3, where 0 = no symptoms, 1 = lesions on lateral branches only, 2 = lesions on the main stem but little or no effects on pod fill, and 3 = lesions on main stem resulting in plant death or poor pod fill. A disease severity index (DSI) was calculated for each plot using the formula: DSI=100 x (Σ r/3n) (r = rating of each plant; n = number of plants rated). The DSI ranges from 0 for absence of disease to 100 for severe infection. Before harvesting, plant height and lodging scores were collected. Plant height was determined as the average length of plants in a plot from the ground to the uppermost node of the plant at maturity. Lodging notes were recorded on a scale of 1 to 5 according to the following criteria: 1.0 - almost all plants erect; 2.0 - either all plants leaning slightly, or a few plants down; 3.0 - either all plants leaning moderately (45°), or 25 to 50% down; 4.0 - either all plants leaning considerably, or 50 to 80% down; 5.0 - all plants down.

In addition to field evaluation, the mapping population was tested for Sclerotinia stem rot resistance in greenhouse using a petiole inoculation method described by Del Rio et al. (2001). The *S. sclerotiorum* isolate Montcalm collected from local common beans field was used for the inoculation. The culture was maintained by sub-culturing every three weeks at room temperature on potato dextrose agar (PDA, Sigma® P-2182) medium. A single mycelia plug cut from the culture with 6-mm diameter cork-borer was placed in the center of a new plate with roughly 4 mm thick layer of PDA (39.0 g PDA/L) to produce inocula for the inoculation. Each seeded Petri plate was sealed and incubated for three days

at 22 °C with 10 hours light daily. A mycelia plug on the growing edge of the 3-day old *S. sclerotiorum* colonies was loaded at the wide end of a 1 ml pipette tip (USA Scientific, Ocala, FL) by pushing the wide end of the tip through the media. The first fully open trifoliate leaf was removed with a razor blade approximately 2.5 cm above the point of attachment to the petiole. The cut petiole was forced into the wide end of the pipette tip containing the mycelial plug to ensure contact of the cut petiole surface with the mycelia. Ten plants of each F_4-derived line were inoculated. The number of plants wilted during each day in the 10 days following inoculation was recorded. AUDPC (Area Under Disease Progress Curve) was calculated for each line according to the formula of Shaner and Finney (1977).

Linkage and QTL analysis

The computer program JoinMap 3.0 (Van Ooijen and Voorrips, 2001) and the Kosambi mapping function (Kosambi, 1944) were used to determine the linkage relationships among the polymorphic SSR markers. The likelihood of odds (LOD) groupings threshold was set to 3.0 to divide markers into linkage groups. The best position of each marker was searched by comparing the goodness-of-fit for each tested position to determine the order and distance among markers within each linkage group. The linkage map resulting from JoinMap analysis was used as map input in the QTL analysis using WinQTLcart V2.5 (Wang et al., 2005).

A simple linear regression model from WinQTLcart V2.5 was used to identify markers significantly (P value = 5%) associated with Sclerotinia stem rot resistance. Model

6 was used with window size 10 cM and 5 background markers. The LOD threshold used to declare the putative QTLs significant in composite interval mapping (CIM) method was determined by 1000 permutations (Churchill and Doerge, 1994). The estimated QTL position was at the peak position of LOD score curve.

RESULTS

Phenotypic Data

Field DSI from Illinois in 1998 and 1999 were approximately normally distributed with a range from 0 to 65 (Figure 3-1). The distributions of field DSI from Michigan in 1998 and 1999 were skewed to the right with a range from 0 to 70. The 2002 field DSI was only collected from Michigan and DSI distribution was severe skewed to the right with a range from 0 to 35 (Figure 3-1). For the parents, the DSI for Asgrow 2506 was 16 and for NKS 19-90 was 0. In 2002, the field condition was hot and dry which was not suitable for Sclerotinia stem rot development.

Greenhouse average AUDPC in 2002 (Figure 3-2) was approximately normally distributed with a range from 0 to 16. For the parents, the AUDPC for Asgrow 2506 was 14 and for NKS 19-90 was 0. However, the distribution of the average AUDPC in 2003 (Figure 3-2) was slightly left skewed with a range from 2 to 7. For the parents, the AUDPC for Asgrow 2506 was 5 and for NKS 19-90 was 2. In the 2003 greenhouse experiments the inoculated plants were younger than those in the 2002 experiments in which plants in 2003 were early V2 stage but plants in 2002 were late V2 stage so the disease progressed faster

in 2003. Over 80% of the inoculated plants were dead on the 10^{th} day after inoculation in 2003, while only 40% of the inoculated plants were dead on the 10^{th} day after inoculation in 2002.

Figure 3-1.

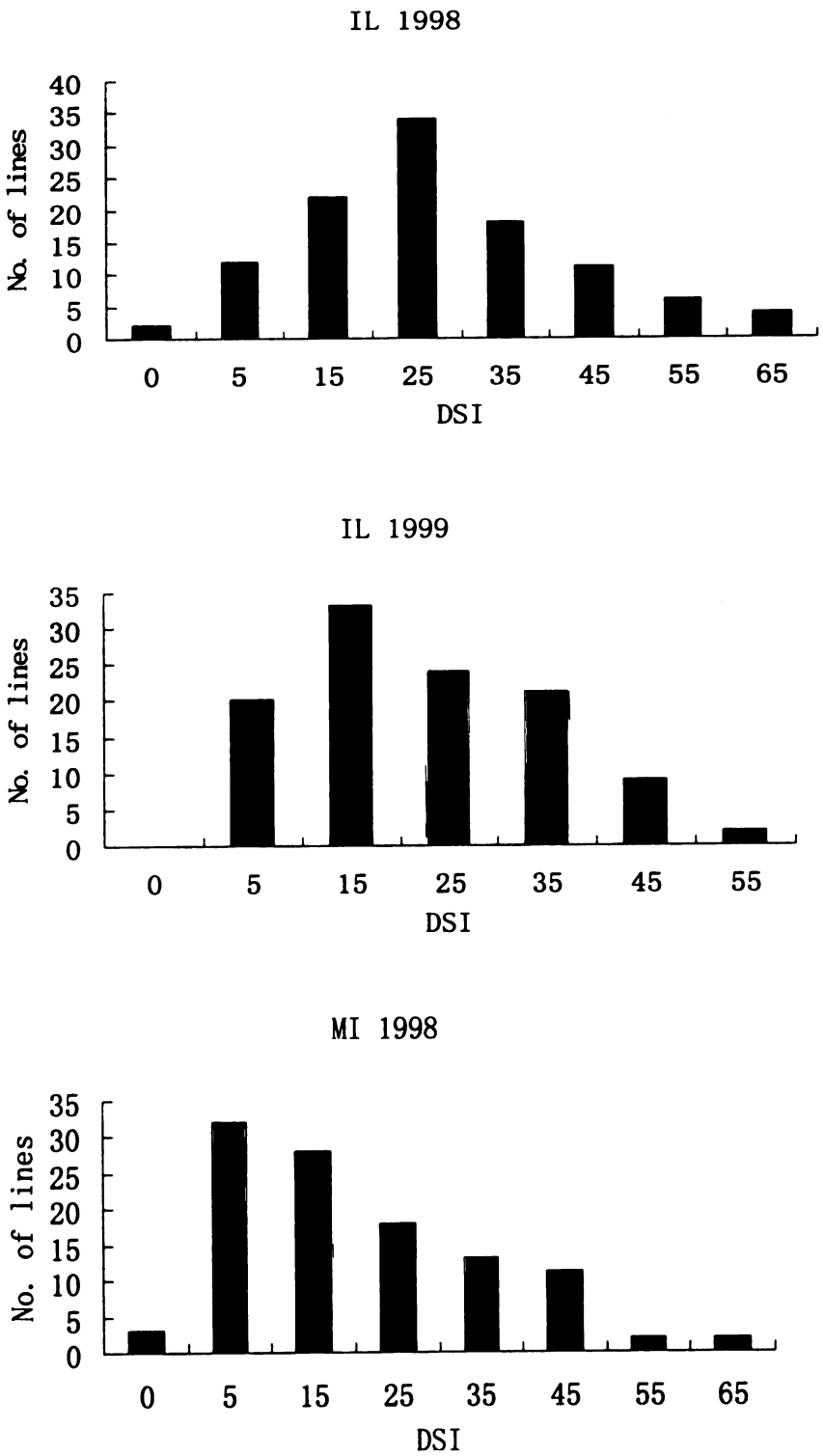
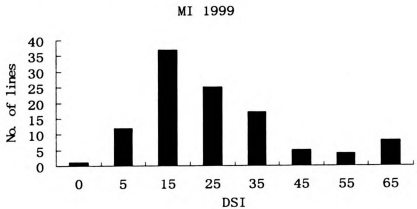
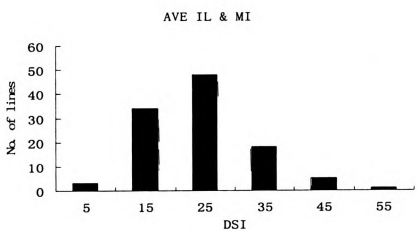

IL 1998
No. of lines
40
35
30
25
20
15
10
5
0
0
5
15
25
35
45
55
65
DSI
IL 1999
No. of lines
35
30
25
20
15
10
5
0
0
5
15
25
35
45
55
DSI
MI 1998
No. of lines
35
30
25
20
15
10
5
0
0
5
15
25
35
45
55
65
DSI

Figure 3-1. (cont'd)





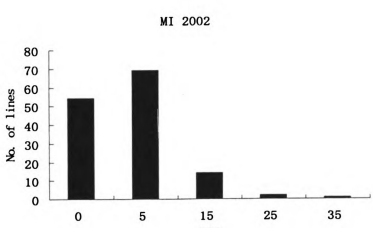


Figure 3-1. The DSI distributions of 140 F_4–derived lines from a cross between Asgrow 2506 and NKS 19-90 in 1998, 1999, and 2002 field experiments. The DSI was the average of the two replications with 30 plants in each replication.

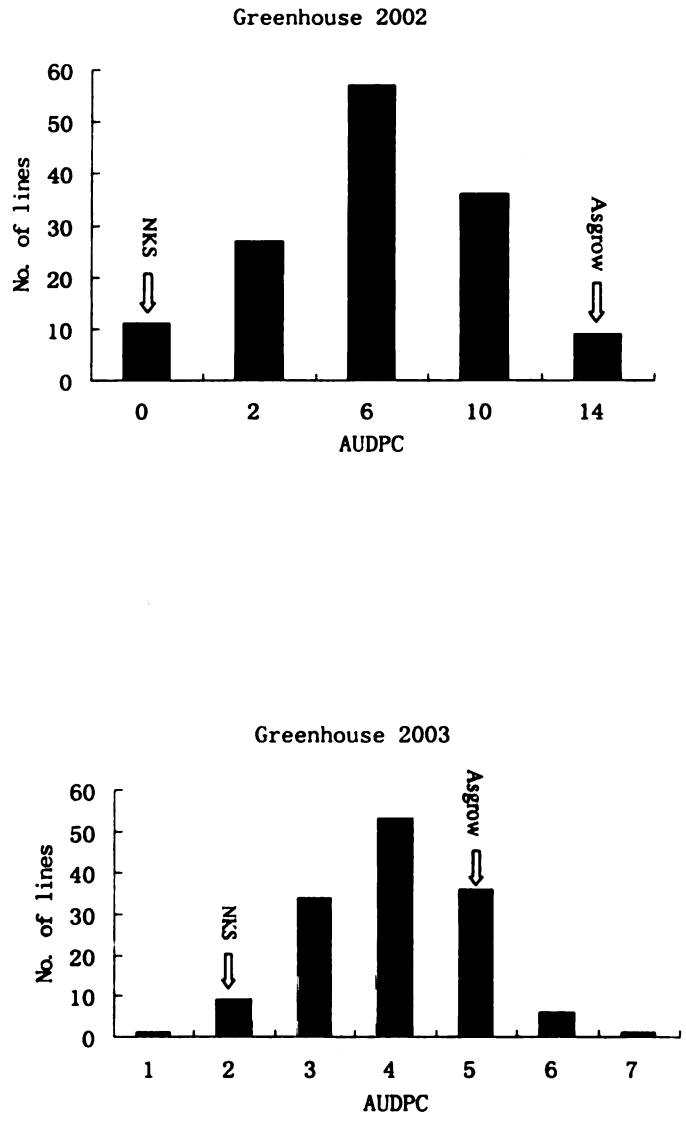


Figure 3-2. Distributions of the average AUDPC of 140 F4–derived lines from a cross between Asgrow 2506 and NKS 19-90 in the 2002 and 2003 greenhouse experiments. The plants at late and early V2 stages in 2002 and 2003, respectively, were inoculated with the cut-petiole method. The AUDPC depends on the percentage of wilted plants and the observation days after inoculation. The observation period was 20 and 10 days after inoculation in 2002 and 2003, respectively. The AUDPC was calculated based on 10 plants in each replication with four replications.

The broad-sense heritability estimates were obtained by proc glm in SAS analysis. The heritability was 0.41 in 1998 and 1999 in Michigan and Illinois field data and it was 0.24 and 0.16 in 2002 and 2003 greenhouse environment in Michigan, respectively.

Identification of QTL for Sclerotinia stem rot resistance

Of the 1132 SSR markers tested for polymorphism between the two parents, only 53 were polymorphic. The 53 polymorphic markers were placed into 9 linkage groups with a LOD threshold of 3.0 using Joinmap 3.0. The 9 linkage groups corresponded with soybean composite map (Song et al., 2004) linkage groups A1, B2, C1, D1b, D2, D1a and G, H, N, and O. The total map distance of the 9 linkage groups with 25 SSR markers was 116.2 cM with average interval length 4.6 cM. However, the total map distance was only 49.6 cM using Song et al. (2004) map and it was only 2% of the soybean genome coverage.

Both single marker analysis (SMA) and composite interval mapping (CIM) methods were used in the QTL analysis. Markers from different linkage groups were significantly associated with soybean Sclerotinia stem rot resistance but only the marker Sat_327 from linkage group C1 was relatively consistent among the tests in different years. Single marker analysis (Table 3-1) identified marker Sat_327 on linkage group C1 significantly ($P<0.001$) associated with soybean Sclerotinia stem rot resistance in field test in Michigan in 1999 and in greenhouse test in 2003. The resistance allele was from NKS 19-90. Marker-wise P=0.001 is equivalent to experiment-wise P=0.001*53 (polymorphic markers) =0.05 in my study. The CIM method also identified a QTL (Figure 3-3) around marker Sat_327 with LOD scores of 9.2 and 3.7 comparing to 3.4 and 3.5 of the LOD thresholds

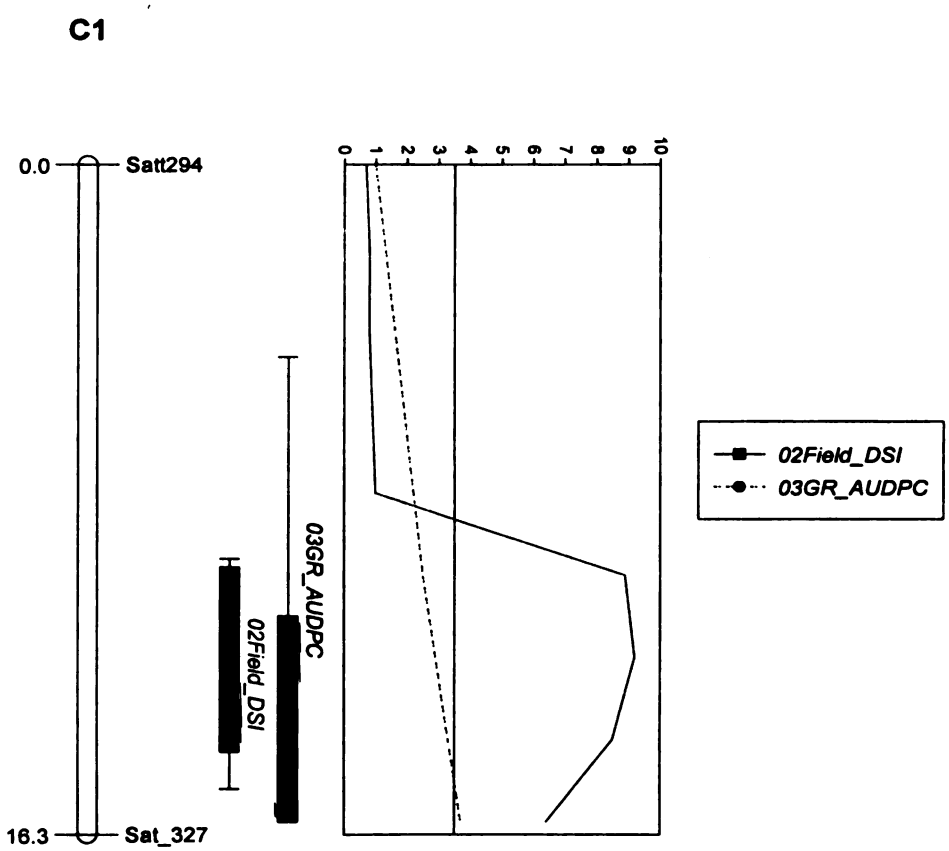
for $P \leq 0.05$ determined by 1000 permutations for the 2002 Michigan field tests and the AUDPC of the 2003 greenhouse test, respectively (Figure 3-3). The QTL explained 62.1% and 12.2% of the total phenotypic variances of the 2002 Michigan field tests and the AUDPC of the 2003 greenhouse test, respectively.

Table 3-1. Markers significantly associated with Sclerotinia stem rot resistance at the 0.01 probability level at least in one environment in single marker analysis. Markers significant at the 5%, 1%, 0.1% and 0.01% levels were indicated by *, **, ***, and **** respectively. Linkage group names and relative position for the markers were assigned according to the Soybean Composite Map (Song et al., 2004).

Marker Name	LG †	Position ‡	p-value						Source of R Allele
			MI Field 1998	MI Field 1999	AVE 98 & 99	MI Field 2002	GH§ 2002	GH 2003	
Sat_327	C1	?	0.003 **	0.001 ***	0.002 **	0.019 *	0.016 *	0.000 ****	NKS 19-90
Satt371	C2	145.5	0.008 **	NS	NS	NS	NS	NS	A2506
Satt463	M	50.1	NS	NS	NS	NS	0.009 **	NS	NKS 19-90
Satt445	O	20.4	NS	NS	NS	NS	0.001 **	NS	NKS 19-90
Satt526	A1	28.0	NS	NS	NS	NS	0.006 **	NS	NKS 19-90
Sctt008	D2	3.2	NS	NS	NS	NS	NS	0.006 **	NKS 19-90
Satt373	L	107.2	NS	NS	NS	NS	NS	0.005 **	NKS 19-90

† LG = Linkage Group
‡ "?" means no position available on the soybean composite map.
§ GH = Greenhouse; NS = not significant at 0.05 probability level.

Figure 3-3. Putative QTL associated with Sclerotinia stem rot resistance on linkage group C1 and the map distances between the markers are given in cM (centiMorgans). The linkage group was named according to Song et al. (2004). A QTL was identified around SSR marker Sat_327 in 2002 field and 2003 greenhouse environments. The LOD§ threshold after 1000 permuatation was 3.4 and 3.5, respectively.



§ The LOD threshold in the figure was 3.5.

The correlation between DSI and plant height was 0.26 ($p<0.01$) and the correlation between DSI and lodging score was 0.31 ($p<0.001$) in 1998 and 1999. However, no significant association was identified between Sclerotinia stem rot resistance with height and lodging in SMA and QTL analysis.

DISCUSSION

The results obtained indicate that the allele from linkage group C1 contribute to soybean Sclerotinia stem rot resistance in NKS 19-90. A QTL region near Sat_327 was identified by SMA and CIM in both field and greenhouse experiments. Previous studies (Arahanna et al., 2001; Kim and Diers, 2000) did not identify any markers from linkage group C1 significantly associated with soybean Sclerotinia stem rot resistance. The resistance allele at the marker Sat_327 was from soybean variety NKS 19-90. The reason that no resistance allele from Asgrow 2506 was idientified might be that the resistance alleles in Asgrow 2506 are also present in NKS 19-90 so there was no segregation for these alleles in the mapping populations. Another reason could be that there were no polymorphic markers near the resistance allele in Asgrow 2506. A third possible reason could be that the phenotypic data were collected under conditions not favouring the expression of the resistance allele in Asgrow 2506.

Most significant markers were not identified by other studies. Arahana et al. (2001) identified some markers using SMA from the same linkage groups such as A1, D2, L, and O but they are over 50 CM away from the significant markers in my study except that marker Sctt008 on linkage group D2 is about 20 cM from Satt458 in their study.

The correlation between disease index, plant height and lodging were observed and some significant markers from disease score were associated with plant height and lodging (Kim and Diers, 2000). However, there was no significant association between marker

Sat_327 with plant height and lodging at 0.05 probability level.

During the QTL mapping of soybean Sclerotinia stem rot resistance in this population, two big challenges were encountered. One was that not enough polymorphic markers were identified between the two parents and the other was the difficulty in obtaining consistent phenotypic data. It is important to have a sufficient number of polymorphic markers and reproducible phenotypic data to identify useful QTL. The disease development depends on the environmental conditions and this make it difficult to obtain consistent phenotypic data. Nevertheless, the new marker found to be associated with Sclerotinia stem rot resistance in this study can be used in marker assisted selection for resistance to the disease in a soybean breeding program.

Some lines showed better resistance than both parents but they were not significantly better at the 0.05 probability level. So it is hard to say that the resistance was increased by combining resistance genes from Asgrow 2506 and NKS 19-90. One reason might be that both parents share some major resistance genes. Another reason might be the population size was not big enough to have some lines with good combination of all the resistance genes from both parents.

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APPENDIX

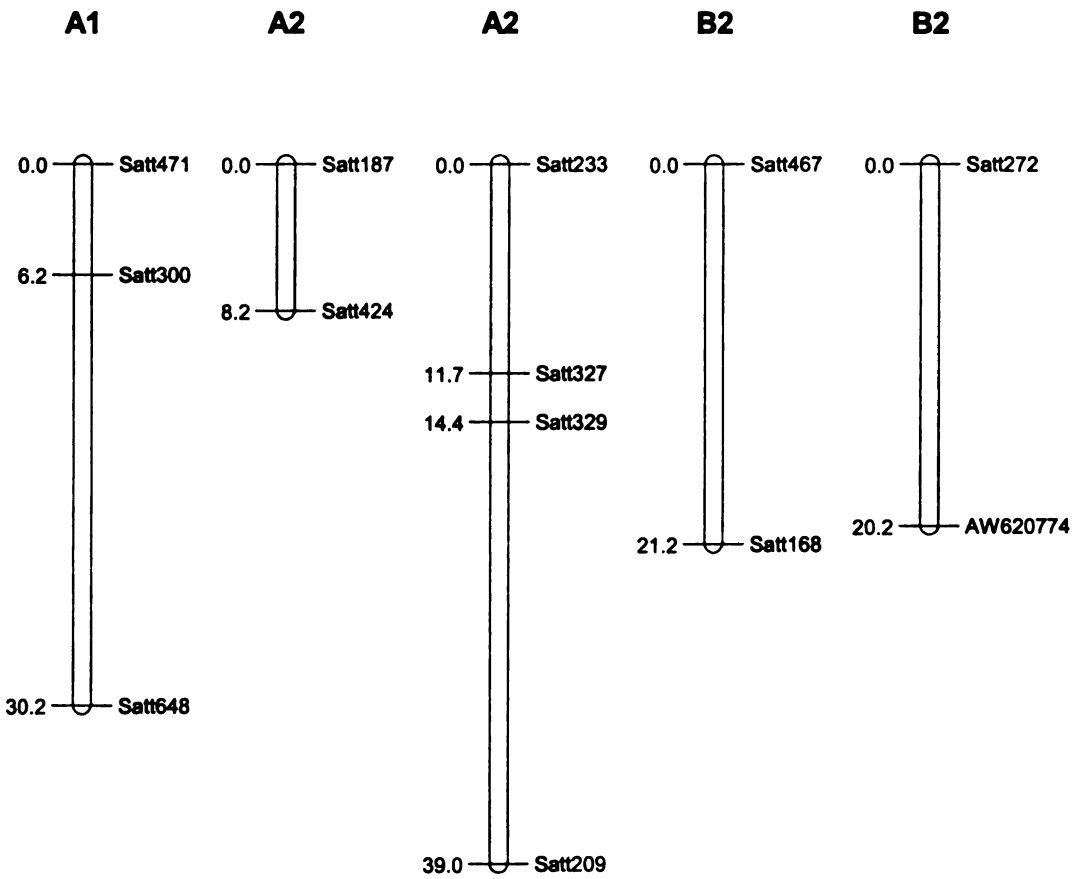


Figure 1. Linkage maps of 94 F_2-derived lines from cross PI 391589 B and IA 2053 constructed using JoinMap 3.0 with a LOD grouping threshold 3.0. The linkage groups were named according to Song et al. (2004) and the map distances between the markers are given in cM (centiMorgans).

Figure 1. (cont'd)

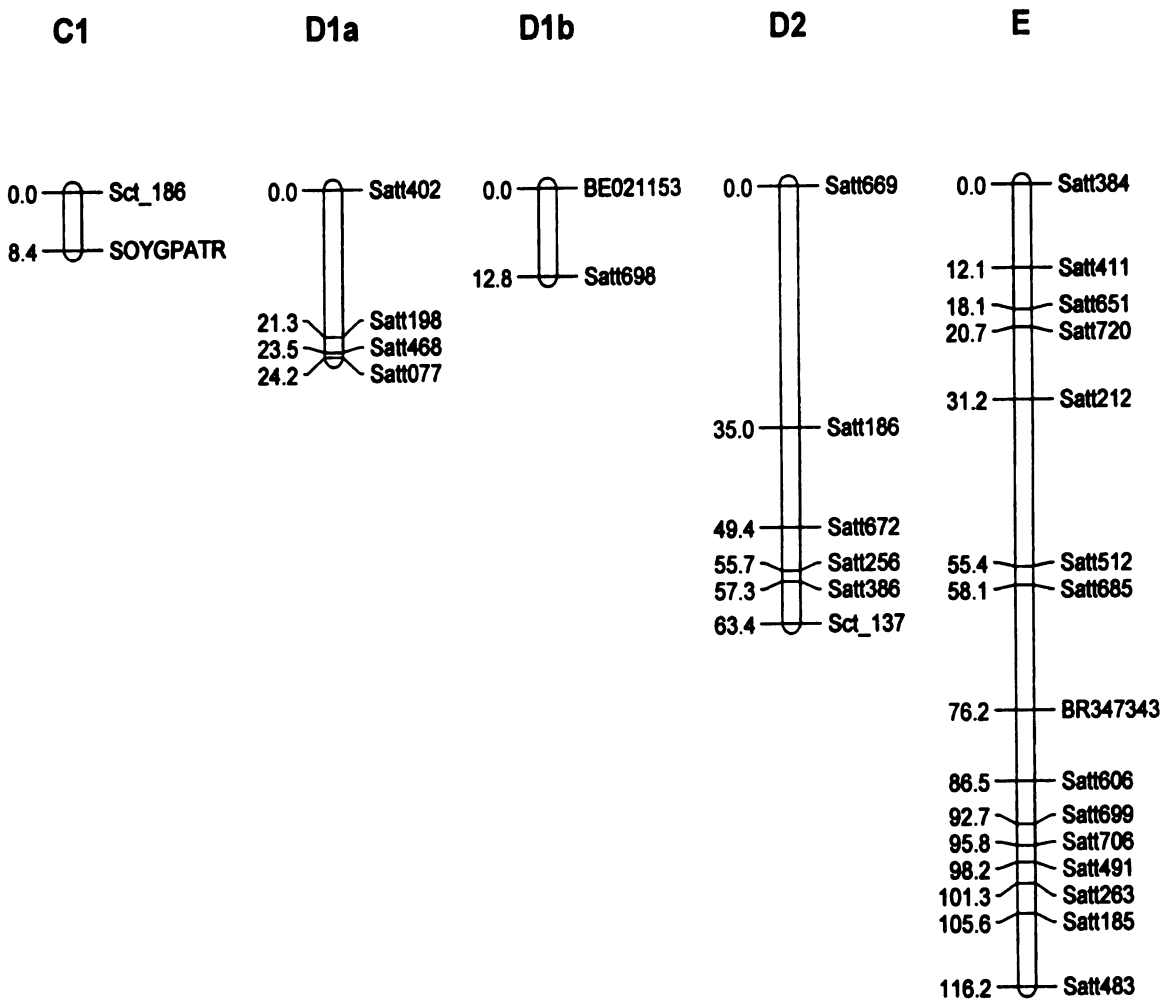


Figure 1. (cont'd)

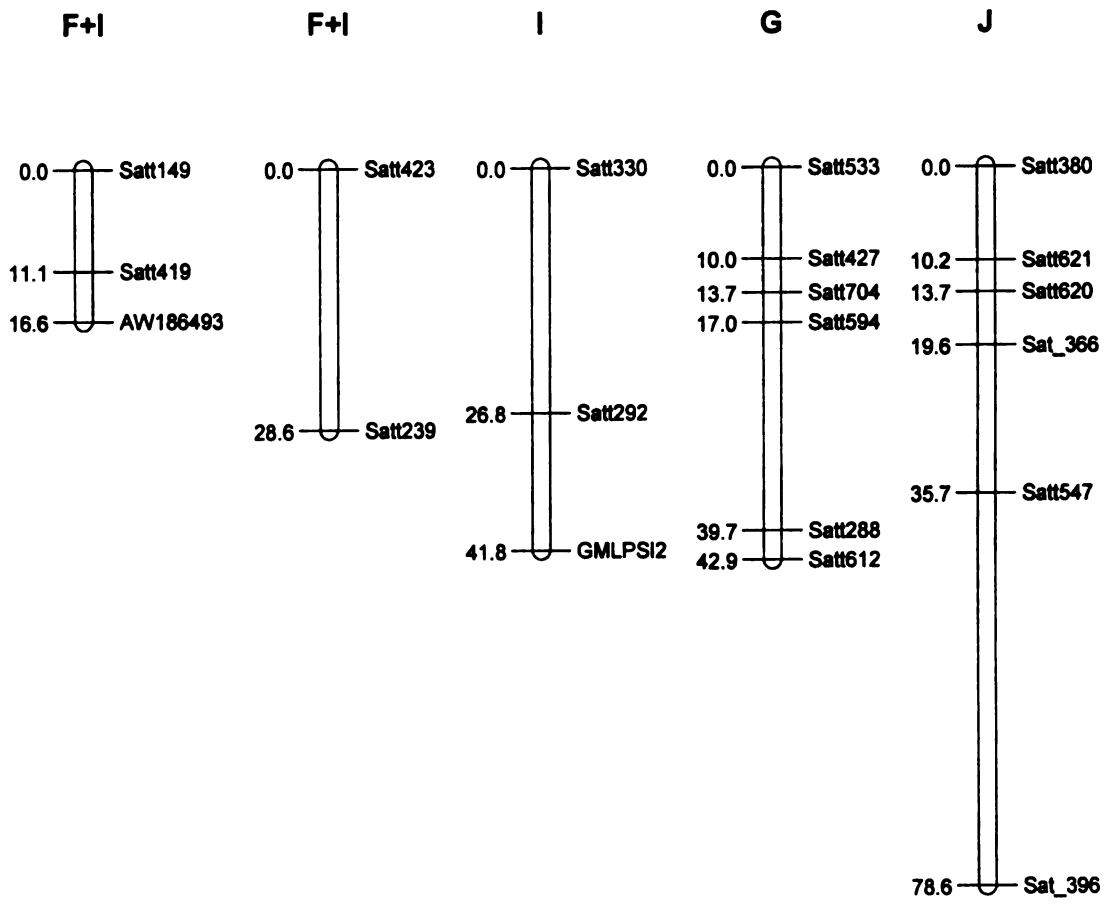


Figure 1. (cont'd)

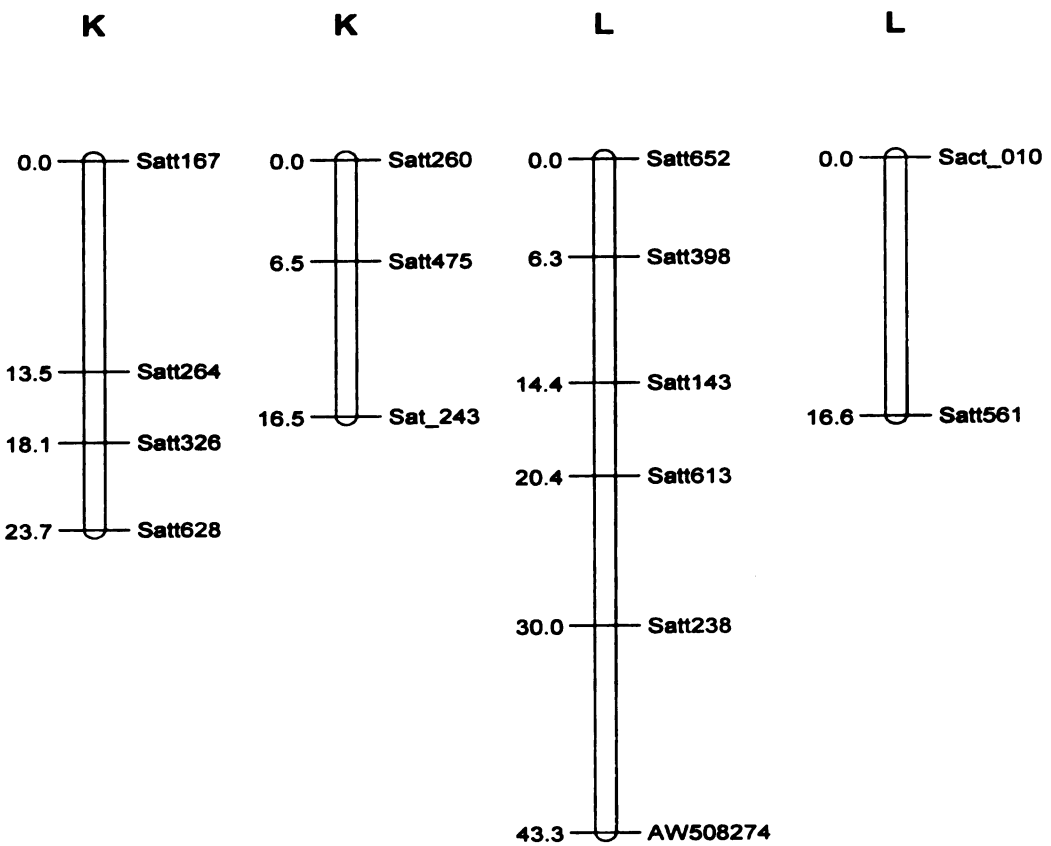
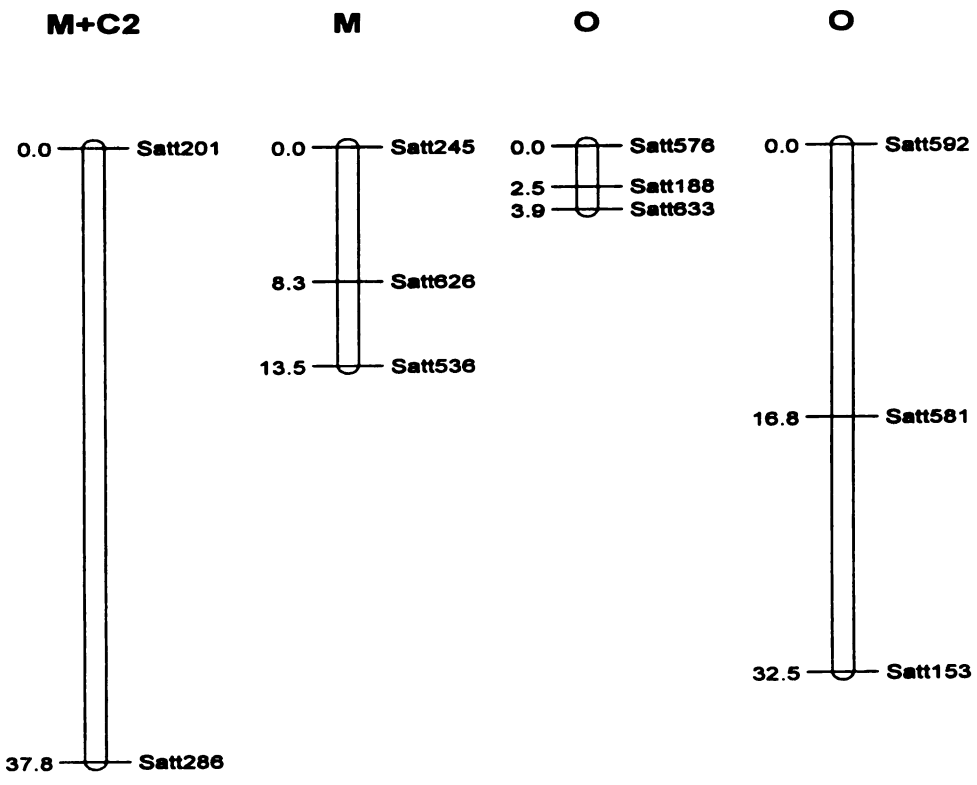


Figure 1. (cont'd)



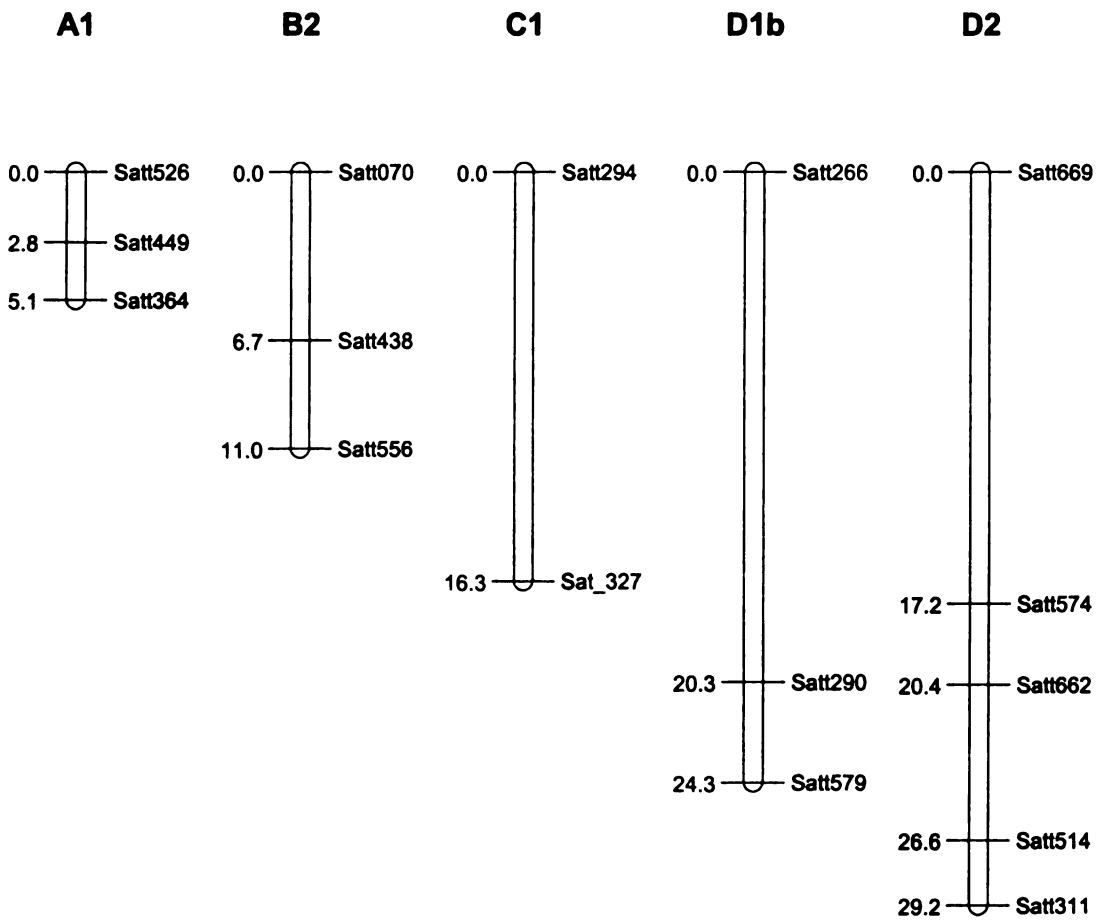


Figure 2. Linkage maps of 140 F_4-derived lines from cross Asgrow 2506 and NKS 19-90 constructed using JoinMap 3.0 with a LOD grouping threshold 3.0. The linkage groups were named according to Song et al. (2004) and the map distances between the markers are given in cM (centiMorgans).

Figure 2. (cont'd)

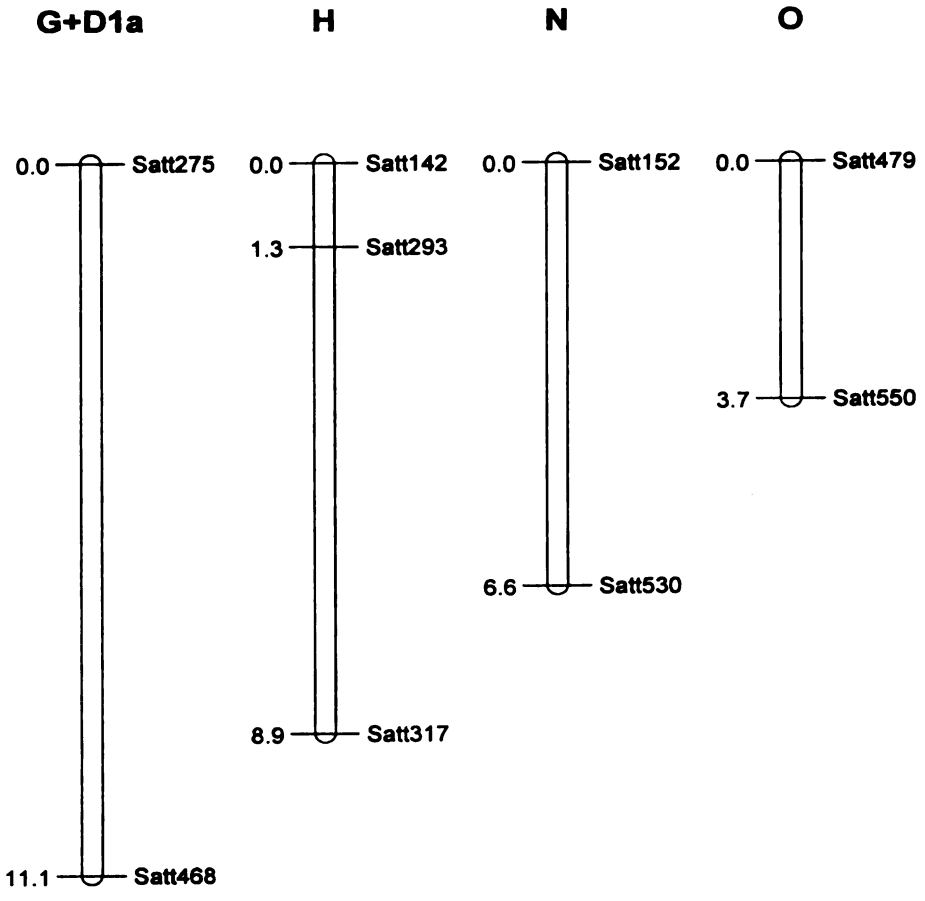


Table 1. Information about all polymorphic simple sequence repeat (SSR) markers from F_2-derived population of PI 391589B X IA 2053

Marker name	Own map (cM)	Integrated map (cM)†	a:h:b	Missing	X^2	Significant level‡
Linkage Group **A1**						
Satt471	0.0	28.0	27:47:17	0	2.3	-
Satt300	6.2	30.9	30:42:19	0	3.2	-
Satt648	30.2	59.2	26:46:17	2	2.0	-
Linkage Group **A2**						
Satt187	0.0	54.9	19:54:18	0	3.2	-
Satt424	8.2	60.6	18:54:19	0	3.2	-
Linkage Group **A2**						
Satt233	0.0	100.1	20:47:24	0	0.5	-
Satt327	11.7	109.9	17:49:25	0	2.0	-
Satt329	14.4	110.9	20:44:26	1	0.9	-
Satt209	39.0	128.4	18:42:31	0	4.2	-
Linkage Group **B2**						
Satt467	0.0	17.8	24:51:16	0	2.7	-
Satt168	21.2	55.2	19:57:15	0	6.1	*
Linkage Group **B2**						
Satt272	0.0	71.7	20:50:19	2	1.4	-
AW620774	20.2	90.3	18:50:19	4	2.0	-
Linkage Group **C1**						
Sct_186	0.0	9.0	37:29:16	9	17.3	***
SOYGPATR	8.4	10.3	26:39:25	1	1.7	-
Linkage Group **D1a**						
Satt402	0.0	57.8	16:42:32	1	6.0	-
Satt198	21.3	68.6	16:52:23	0	2.9	-
Satt468	23.5	69.9	16:50:25	0	2.7	-
Satt077	24.2	77.5	16:47:24	4	2.1	-
Linkage Group **D1b**						
BE021153	0.0	30.2	19:44:27	1	1.5	-
Satt698	12.8	38.0	29:36:25	1	3.9	-

Table 1. (cont'd)

Marker name	Own map (cM)	Integrated map (cM)	a:h:b	Missing	X^2	Significant level
Linkage Group **D2**						
Satt669	0.0	67.7	51:11:25	4	62.1	***
Satt186	35.0	105.5	22:45:24	0	0.2	-
Satt672	49.4	115.0	20:45:22	4	0.3	-
Satt256	55.7	124.3	22:49:19	1	1.0	-
Satt386	57.3	125.0	22:51:18	0	1.7	-
Sct_137	63.4	129.0	19:56:14	2	6.4	*
Linkage Group **E**						
Satt384	0.0	19.3	48:19:23	1	42.6	***
Satt411	12.1	12.9	27:39:24	1	1.8	-
Satt651	18.1	32.1	14(a):60(c)	17	1.5	-
Satt720	20.7	20.8	22:42:26	1	0.8	-
Satt212	31.2	32.3	28:43:20	0	1.7	-
Satt512	55.4	?	23:39:28	1	2.2	-
Satt685	58.1	56.7	24:21:24	22	10.3	**
BR347343	76.2	?	25:44:21	1	0.5	-
Satt606	86.5	39.8	29:34:14	14	6.8	*
Satt699	92.7	41.2	29:45:17	0	3.2	-
Satt706	95.8	43.4	28:38:25	0	2.7	-
Satt491	98.2	43.6	30:37:19	5	4.4	-
Satt263	101.3	45.4	36:36:19	0	10.1	**
Satt185	105.6	44.8	30:36:24	1	4.4	-
Satt483	116.2	45.0	20:40:29	2	2.7	-
Linkage Group **F+I**						
Satt149	0.0	18.1(F)	26:33:32	0	7.5	*
Satt419	11.1	21.9(I)	29:38:23	1	3.0	-
AW186493	16.6	21.0(F)	32:34:22	3	6.7	*
Linkage Group **F+I**						
Satt423	0.0	20.6(F)	27:46:17	1	2.3	-
Satt239	28.6	36.9(I)	24:48:16	3	2.2	-
Linkage Group **I**						
Satt330	0.0	77.8	21:9:61	0	68.1	***
Satt292	26.8	82.8	22:48:20	1	0.6	-
GMLPSI2	41.8	97.0	22:45:21	3	0.2	-

Table 1. (cont'd)

Marker name	Own map (cM)	Integrated map (cM)	a:h:b	Missing	X^2	Significant level
Linkage Group **G**						
Satt533	0.0	56.5	23:37:28	3	2.8	-
Satt427	10.0	51.7	26:39:25	1	1.7	-
Satt704	13.7	?	28:39:23	1	2.2	-
Satt594	17.0	52.9	25:40:23	3	0.9	-
Satt288	39.7	76.8	18:44:21	8	0.6	-
Satt612	42.9	80.4	18:50:21	2	1.6	-
Linkage Group **J**						
Satt380	0.0	43.1	19:52:20	0	1.9	-
Satt621	10.2	?	19:41:26	5	1.4	-
Satt620	13.7	?	21:47:23	0	0.3	-
Sat_366	19.6	52.7	21:42:27	1	1.3	-
Satt547	35.7	67.7	22:45:23	1	0.1	-
Sat_396	78.6	69.0	4:17:70	0	127.3	***
Linkage Group **K**						
Satt167	0.0	45.7	34:23:19	15	17.2	***
Satt264	13.5	46.2	24:42:25	0	0.6	-
Satt326	18.1	49.5	24:46:21	0	0.3	-
Satt628	23.7	49.6	18:46:21	6	0.9	-
Linkage Group **K**						
Satt260	0.0	80.1	14:51:23	3	4.0	-
Satt475	6.5	78.7	16:46:27	2	2.8	-
Sat_243	16.5	86.8	23(b):57(d)	11	0.7	-
Linkage Group **L**						
Satt652	0.0	30.9	23:20:13	35	7.9	*
Satt398	6.3	30.6	19:47:25	0	1.0	-
Satt143	14.4	30.2	31:38:17	5	5.6	-
Satt613	20.4	36.1	26:36:21	8	2.1	-
Satt238	30.0	19.9	24:48:19	0	0.9	-
AW508274	43.3	38.8	30:42:16	3	4.6	-
Linkage Group **L**						
Sct_010	0.0	59.5	31:40:19	1	4.3	-
Satt561	16.6	71.4	28:36:17	10	4.0	-
Linkage Group **M+C2**						
Satt201	0.0	13.6(M)	26:47:17	1	2.0	-
Satt286	37.8	101.8(C2)	20:52:19	0	1.9	-

Table 1. (cont'd)

Marker name	Own map (cM)	Integrated map (cM)	a:h:b	Missing	X^2	Significant level
Linkage Group **M**						
Satt245	0.0	53.5	24:36:27	4	2.8	-
Satt626	8.3	58.6	19:44:17	11	0.9	-
Satt536	13.5	62.1	21:43:25	2	0.5	-
Linkage Group **O**						
Satt576	0.0	55.8	22:47:21	1	0.2	-
Satt188	2.5	55.6	22:45:19	5	0.4	-
Satt633	3.9	56.9	25:45:20	1	0.6	-
Linkage Group **O**						
Satt592	0.0	100.4	26:46:18	1	1.5	-
Satt581	16.8	106.0	27:36:23	5	2.7	-
Satt153	32.5	128.4	24:46:20	1	0.4	-
Unlinked Group						
Sat_217		101.6(A1)	20:46:21	4	0.3	-
Satt359		102.6(B1)	24:39:28	0	2.2	-
Satt294		78.6 (C1)	19:49:23	0	0.9	-
Satt476		80.6 (C1)	17:45:15	14	2.3	-
Satt338		123.8 (C1)	26:50:15	0	3.6	-
Satt227		26.6 (C2)	15:53:23	0	3.9	-
Satt422		44.7 (C2)	24:42:25	0	0.6	-
Satt322		82.2 (C2)	15:53:23	0	3.9	-
Satt433		128.2 (C2)	27:46:18	0	1.8	-
Satt408		14.2(D1a)	24:48:19	0	0.8	-
Satt353		8.5 (H)	32:32:27	0	8.6	**
Satt541		53.4 (H)	22:47:22	0	0.1	-
Satt674		16 (J)	18:34:12	27	1.4	-
Satt715		0.9 (K)	21:50:20	0	0.9	-
Satt513		106.4 (L)	65:0:25	1	125.6	***
Sct_195		2.4 (N)	26:35:17	13	2.9	-
Satt358		5.4 (O)	20:49:21	1	0.7	-
Satt445		20.4 (O)	3:29:58	1	78.6	***
Satt054		?	16:42:27	6	2.9	-
Satt059		?	17:45:29	0	3.2	-
Satt108		?	23:42:26	0	0.7	-

† "?" means no position available on the soybean composite map.

‡ "-" means not significant at 0.05 probability level; *, **, and *** means Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 2. Information about all polymorphic simple sequence repeat (SSR) markers from F_4-derived population of Asgrow 2506 X NKS 19-90

Marker name	Own map (cM)	Integrated map (cM)†	a:h:b	Missing	X^2	Significant level‡
Linkage Group **A1**						
Satt526	0.0	28.0	68:5:63	4	9.9	**
Satt449	2.8	27.8	64:17:56	3	0.5	-
Satt364	5.1	29.0	64:11:61	4	2.5	-
Linkage Group **B2**						
Satt070	0.0	72.8	61:8:64	7	5.2	-
Satt438	6.7	?	67:21:50	2	3.3	-
Satt556	11.0	73.2	76:7:53	4	11.2	**
Linkage Group **C1**						
Satt294	0.0	78.6	44:35:58	3	23.0	***
Sat_327	16.3	?	66:0:60	14	18.3	***
Linkage Group **D1b**						
Satt266	0.0	59.6	81:6:53	0	15.0	***
Satt290	20.3	73.3	67:5:57	11	9.7	**
Satt579	24.3	75.9	72:16:50	2	4.1	-
Linkage Group **D2**						
Satt669	0.0	67.7	66:9:63	2	4.6	-
Satt574	17.2	87.7	59:9:70	2	5.5	-
Satt662	20.4	87.9	61:12:61	6	1.5	-
Satt514	26.6	85.7	56:35:49	0	20.4	***
Satt311	29.2	84.6	50:33:56	1	16.4	***
Linkage Group **G+D1a**						
Satt275	0.0	2.2 (G)	53:15:72	0	3.4	-
Satt468	11.1	69.9(D1a)	55:12:73	0	4.6	-
Linkage Group **H**						
Satt142	0.0	86.5	65:4:71	0	12.2	**
Satt293	1.3	89.1	65:4:68	3	11.6	**
Satt317	8.9	89.5	64:9:63	4	4.3	-
Linkage Group **N**						
Satt152	0.0	22.7	58:2:65	15	14.0	***
Satt530	6.6	32.8	66:8:63	3	5.6	-
Linkage Group **O**						
Satt479	0.0	54.2	56:2:80	2	20.2	***
Satt550	3.7	55.1	50:10:77	3	9.5	**

Table 2. (cont'd)

Marker name	Own map (cM)	Integrated map (cM)	a:h:b	Missing	X^2	Significant level
Unlinked Group						
Satt545		71.4 (A1)	66:15:58	1	0.9	-
GMENOD2B		58.4 (A2)	68:0:55	17	19.1	***
Satt409		145.6(A2)	68:1:49	22	18.1	***
Satt560		97.9 (B2)	76:5:59	0	12.6	**
Satt180		127.8(C1)	55:3:69	13	13.7	**
Satt316		127.7(C2)	63:23:54	0	2.6	-
Satt371		145.5(C2)	54:26:56	4	5.5	-
Satt198		52.3(D1a)	65:20:55	0	1.2	-
Sctt008		3.2 (D2)	56:17:67	0	1.0	-
Satt002		47.7 (D2)	62:5:50	23	8.6	*
Satt464		89.8 (D2)	67:19:49	5	3.0	-
Satt252		16.1 (F)	60:14:65	1	1.0	-
Satt114		63.7 (F)	63:11:65	1	2.7	-
Satt570		12.7 (G)	52:2:51	35	10.8	**
Satt115		43.8 (G)	58:8:65	9	5.3	-
Satt288		76.8 (G)	64:11:57	8	2.5	-
Satt451		20.3 (I)	55:8:73	4	8.2	*
Satt475		78.7 (K)	56:11:73	0	5.1	-
Satt523		27.9 (L)	70:16:54	0	2.2	-
Satt448		64.7 (L)	66:10:61	3	3.6	-
Satt373		107.2 (L)	60:24:49	7	4.8	-
Satt463		50.1 (M)	61:14:60	5	0.6	-
Satt308		130.8 (M)	77:9:46	8	12.2	**
Satt257		92.6 (N)	67:8:59	6	5.8	-
Satt445		20.4 (O)	69:13:57	1	2.4	-
Satt120		?	58(b):77(c)	5	0.0	-
Satt512		?	71:10:59	0	4.8	-
Sat_146		?	73:4:56	7	13.4	**

† "?" means no position available on the soybean composite map.

‡ "-" means not significant at 0.05 probability level; *, **, and *** means Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.