MOLECULAR CHARACTERIZATION OF GENETIC RESISTANCE TO SOYBEAN CYST NEMATODE IN SOYBEAN LINE SS97-6946

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

MOLECULAR CHARACTERIZATION OF GENETIC RESISTANCE TO SOYBEAN CYST NEMATODE IN SOYBEAN LINE SS97-6946

Presented by Md Sariful Islam,

A candidate for the degree of Master of Science

And hereby certify that, in their opinion, it is worthy of acceptance.

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Molecular Characterization of Genetic Resistance to Soybean Cyst Nematode in Soybean Line SS97-6946

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Abstract

Soybean cyst nematode (SCN) *Heterodera glycines* Ichinohe is the most yield reducing pest of soybean and estimated yield losses are 1.5 billion dollars annually in USA. Breeding resistant cultivars is the most efficient means to control SCN, but the nematode has adapted and overcome resistance of developed soybean cultivars due to narrow genetic base. Resistant genes for different nematode HG types have been genetically mapped in several soybean accessions, but most of them have common quantitative trait loci (QTL). However, *H. glycines* populations are enormously variable and have broken down resistance ability of cultivars. Eventually cultivars become less effective in reducing yield losses. Hence, it is essential to explore new soybean SCN resistant sources for discovery of new genes that confer resistance to SCN field populations to offer long-lasting resistance. Soybean line SS97-6946 is resistant to different SCN HG types and could have novel genes for SCN resistance. Objectives of this study were to investigate the genetic basis of resistance to SCN populations of HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (corresponding to races 1, 2, 3, and 5, respectively) and determine the QTL associated with resistance to SCN populations of HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (corresponding to races 1, 2, 3, and 5, respectively) in SS97-6946. PI 567476, moderately susceptible to SCN races was crossed with SS97-6946 to produce F_1. One hundred sixty F_2 and $F_{2:3}$

families were developed to investigate the reaction to specific SCN HG types in the greenhouse. Three, two, 24, and seven $F_{2:3}$ families were categorized as resistant (FI≤10) to SCN races 1, 2, 3, and 5, respectively. On the other hand 157, 158, 136, and 153 individuals showed susceptible (FI>10) reaction to these four SCN races, respectively. Ratio of resistant to susceptible $F_{2:3}$ families fitted a three-gene model of inheritance. It was speculated that a genotype with three recessive genes (*rhg, rhg,* and *rhg*) for races 1 and 2; two dominant and one recessive (*Rhg, Rhg,* and *rhg*) for race 3; one dominant and two recessive (*Rhg, rhg,* and *rhg*) for race 5 existed. Some $F_{2:3}$ families had broad-spectrum resistance to multiple SCN races due to all tested SCN HG types being highly correlated. Three-hundred forty-seven out of 547 (<63%) SSR primer pairs were found polymorphic between PI 567476 and SS97-6946. These selected 347 polymorphic primer pairs covered all 20 linkage group of soybean to screen 160 F_2 families derived from the cross PI 567476 X SS97-6946. Phenotypic and genotypic data were analyzed to identify markers associated with resistant QTL to SCN by using MapQTL software. Markers SUIC 100-8K, Satt268, and Satt610 were mapped on linkage groups (LGs) A2, E and G (chromosome no. 8, 15 and 18), respectively, and were found to be associated with resistance to SCN race 1 and accounted for 29.1 % of the total phenotypic variance. One resistant QTL was detected in LG A1 (Chromosome no. 5) associated with marker Sat_368 and accounted for 18.8% of total phenotypic variance of race 2. Markers SUIC 100-8K, Satt610, and Satt551 on LGs A2, G, and M (chromosome no. 8, 18 and 7), respectively were discovered to be associated with resistance to SCN race 3 and shared 30.5% of total phenotypic variance. Markers Sct_187 and Satt610 on LG G (chromosome no. 18) together with markers Sat_368, Satt 665, Sat_287, Sat_121, and Satt173 on LG

A1, B1, B2, M, and O (chromosome no. 5, 11, 14, 7 and 10), respectively, were shown to be linked with SCN resistance to race 5 and accounted for 58.9% of total phenotypic variance. Resistance QTL detected in LGs A1, A2, G, M and O could come from resistant parent SS97-6946. Other QTL identified in LGs B1, B2, E could come from PI 567476.

Abbreviations: * Soybean Plant Introduction *Female Index *Quantitative Trait Loci *Simple Sequence Repeat *Linkage Group

Chapter 1

Introduction

Cultivated soybean (*Glycine max L. Merr*) is classified as an important field crop in the United States and in the world in terms of its high nutritional and economic value. The genus *Glycine* has two subgenera; one is perennial *Glycine* and the other is the annual *Soja* (Moench) F. J. Herm. The annual soybean *Soja* is divided into two subfamilies that include cultivated soybean *Glycine max* and wild *Glycine soja* (Hymowitz 2004). Soybean plants are diploid with 20 pairs of chromosomes on which currently 20 linkage groups have been constructed (Hymowitz 2004; Song et al. 2004). Soybean cultivation was first started as a food crop in China 5000 years ago and was introduced into USA in the late 18^{th} century. USA is the number one soybean producer (32%) and exports (37%) of total soybean in the world (Soystats.com, 2008).

Soybean yields are significantly affected by pests like nematodes and other pathogens. Among those, soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe is the most serious pest on cultivated soybean. Wrather et al., (2001, 2006) estimated that soybean yield reduction annually by SCN is approximately 1.5 billion dollars in the USA. Soybean cyst nematode was first discovered in Japan and was documented in the USA in 1954. Soybean cyst nematode is soil borne and a transmitted pest that parasitizes inside the roots by removing plant nutrients, disrupting nutrient and water uptake, retarding root growth (Epps et al., 1962; Doupnik 1993). A 30-day period is needed to complete the SCN life cycle from egg to egg. A wide range of variation is present in SCN in terms of size, color, shape and virulence. Initially a four-race scheme was developed based on differential soybean lines to describe genetic diversity of SCN (Golden et al., 1970).

Later 16 races were designated based on reaction of the nematode to four soybean genotypes (Riggs et al., 1988). But the 16 race scheme was inadequate to show the extensive genetic variability of SCN populations. Niblack et al., (2002) proposed a new classification scheme for more accurate and scientific identification of genetically diverse populations of *H. glycines* instead of the race scheme. In the new scheme, '*Heterodera glycines* (HG) type' was proposed instead of 'race'; and seven soybean indicator lines (Peking, PI 88788, PI 90763, PI 437654, PI 209332, PI 89772 and Cloud) are currently being used in determining HG types (Niblack et al., 2002).

Once established in a field, it is impossible to eradicate SCN by using any recognized economical method (Schumann et al., 2007). Several cultural practices have been used such as crop rotation to reduce losses. Nematicides have been applied to control nematodes in some crops. But nematicides are costly and have restrictions for use in crops due to high toxicity and are environmentally unsafe. Breeding and use of resistant cultivars is the primary and most effective management tool currently being employed to control SCN. Due to extremely variable populations of *H. glycines* (Ross 1962; Miller 1969; Riggs et al., 1988; Arelli et al., 1992) SCN can overcome resistance in cultivars and is capable of reproducing on resistant varieties and making them less effective in reducing yield losses. Use of several resistant varieties and other non-host crops in rotation is the most effective method to reduce yield losses and SCN race shifts.

The existence of an ever changing nematode population dictates that research on resistant variety development using new resistant sources is needed to minimize yield losses by SCN. Several soybean scientists and breeders have devoted their efforts to discover resistant sources to populations of different HG types primarily 0, 1.2.7 and 1.3.6.7 (races

3, 5 and 14, respectively) by periodically evaluating the soybean germplasm since the detection of SCN in USA (Ross et al., 1957; Epps et al., 1972; Anand et al., 1988; Young 1990, 1995). Arelli et al., (1992) identified a total of 118 resistant Plant Introduction (PI) lines with resistance to one or more of those HG types. Arelli et al., (1997, 2000) also bioassayed those 118 accessions to SCN populations of HG types 2.5.7 and 1.2.5.7 (corresponding to races 1 and 2). The genetic base of host resistance in cultivars is narrow in most of the identified SCN resistance sources. Many SCN resistant varieties have been developed in the USA but almost all have resistance genes from Peking to HG types 2.5.7, and 0 (races 1 and 3, respectively) and/or PI88788 to HG types 0, and 1.3.6.7 (races 3 and 14, respectively). The pathogen has adapted and overcome resistance in soybean cultivars due to shifts to virulent HG types and to narrow genetic base (Dong et al., 1997). HG types 2.5.7, 1.2.5.7 and 1.2.7 (races 1, 2, and 5, respectively) have especially become increasingly more damaging to soybean in some states (Niblack et al., 2003, Mitchum et al., 2007). Thus, it is important to identify novel resistance genes from other sources for breeders to incorporate more effective genes into cultivars for resistance to predominant SCN HG types in soybean fields.

Since the measuring scale of SCN resistance, the female index, is a continuous distribution among the accessions in a population derived from SCN resistant x susceptible cross, so the heredity of SCN resistance is multigenic and quantitative (Mansur et al., 1993; Concibido et al., 2004; Lu et al., 2006). Several soybean scientists and breeders reported their work on inheritance of SCN resistance in soybean. Three recessive genes designated as *rhg1, rhg2* and *rhg3* played a vital role in the degree of resistance in soybean cultivar Peking against SCN populations (Caldwell et al., 1960).

Matson et al., (1965) reported that one dominant gene *Rhg4* was associated with resistance to SCN in Peking. Arelli et al., (1992, 1994) found that another dominant gene *Rhg5* was associated with SCN resistance in PI 88788.

Identification, localization and characterization of QTL associated with key agronomic and defensive traits such as SCN resistance can be done efficiently and professionally by using genetic marker technology. A significant number of genome maps have been constructed in soybean since 1990 using genetic markers. The first comprehensive soybean genetic maps were constructed by using restriction fragment length polymorphism (RFLP) markers (Keim et al., 1990; Shoemaker and Olson, 1993). Later scientists paid attention to integrate all other markers such as random amplified fragment length polymorphisms (RAPD), simple sequence repeats (SSR) with RFLP markers during construction of the soybean genome map. Dozens of soybean accessions were used for genetic map construction. Recent development of single nucleotide polymorphism (SNP) markers was used to saturate the soybean genetic map. Song et al., (2004) developed a composite soybean genome map by using more than 1000 SSR markers, 700 RFLPs, hundreds of SNPs and other biochemical and agronomic markers placed on the 20 soybean linkage groups of soybean. This development represents a powerful means to tag genes in soybean genetic research and breeding. Today, SSR markers are popular and widely used in soybean mapping work because they can be mapped to a single locus with each primer pair; also they are easy to use and highly polymorphic. In addition, most scientists are using composite interval mapping (CIM) (Zeng 1993, 1994) methods to detect individual QTL more specifically and efficiently than ANOVA (Analysis of Variance) and IM (interval mapping).

A well developed genetic map covering all LGs allows researchers to accurately assign specific SCN resistance loci in soybean. A total of more than 20 sources were reported for QTLs association with SCN resistance (Concibido et al, 2004; Guo et al., 2005 & 2006a; Lu et al., 2006). QTLs for SCN resistance to HG types 2.5.7, 1.2.5.7, 0, 2.7 and 1.3.6.7 (race 1, 2, 3, 5 and 14, respectively) have been identified in 19 out of 20 soybean LGs except LG D1b from 13 resistance sources (Guo et al., 2006a, Winter et al., 2007a). Those QTLs shared 1 to 91% of total phenotypic variation for SCN resistance (Concibido et al., 2004; Winter et al., 2007a&b; Lu et al., 2006). The common QTL designated as *rhg1* assigned on LG G effects 54% of the total phenotypic variation for resistance in PI 209332 to SCN race 6, 50% for race 3 and 35% for race 1 (Concibido et al., 1996). Separate studies confirmed the QTL on LG G near *rgh1* in soybean accessions Peking, PI 90763, PI 88788, PI 209332, PI 89772, PI 437654 (Concibido et al., 2004), PI404198A, PI 467312, PI 468916 (*G. soja*) (Guo et al., 2006b), PI 464925B (*G. soja*) (Winter et al., 2007a). A second major QTL designated as *Rhg4* was identified on LG A2 for SCN resistance, which affects 15% of total phenotypic variation in PI 209332 for resistance to SCN races (Concibido et al., 1994). Additional studies confirmed the QTL on LG A2 near *Rhg4* locus in soybean accessions Peking, PI 88788, PI 90763, PI 209332, PI 437654 (Concibido et al., 2004).

Although more than a dozen soybean accessions have been mapped for QTLs association with SCN resistance, most of the QTLs were assigned on the same LG regions or closely linked but few of them are confirmed (Concibido et al., 2004, Guo. et al., 2006b). Thus, it is important to identify and confirm novel resistance genes from

other sources for breeders to incorporate more effective genes into cultivars for resistance to predominant SCN HG types in soybean fields.

Objectives

Soybean germplasm line SS97-6946 has shown resistance to all major SCN HG types and has other desirable traits. It has a relative maturity of 4.3 and was developed from the cross between Essex x PI 438503A with yellow seed coat. Zhang et al., (1999) found one of the parent PI 438503A of SS97-6946 showed resistant to all major SCN races and was a different SCN resistance source from known sources like Peking, PI 437654, and PI 438489B. The seed has high protein and oil content, which is valuable for food, feed and industrial applications. These desirable traits and resistance to multiple HG types is positive for using this in soybean breeding programs to introgress SCN resistance into productive varieties. However, identification and characterization of resistance genes for SCN resistance in this germplasm line has not been studied. Objectives were to investigate the inheritance of resistance to SCN populations of HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (corresponding to races 1, 2, 3, and 5, respectively) and determine the QTLs associated with SCN resistance in SS97-6946.

Chapter 2

Materials and Methods

Plant Materials:

Soybean germplasm line SS97-6946 is resistant to multiple SCN races has purple flowers with grey pubescence. PI567476 also has purple flowers with grey pubescence is moderately susceptible to SCN races and it is a low allergen soybean line. The cross between PI567476 and SS97-6946 was made in the summer in 2006 at the Bradford Research and Extension Center (BREC), located near Columbia, Missouri, USA. The F_1 seeds were grown in Costa Rica in 2006 and F_2 seeds were planted at BREC in summer 2007. Adequate amounts of seed were produced by 160 $F_{2:3}$ progenies and used for greenhouse bioassays of SCN HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (races 1, 2, 3 and 5, respectively).

SCN bioassay

The SCN bioassay was performed in the Ashland road greenhouse at the University of Missouri, Columbia, MO, USA following established methods (Arelli et al., 1994, 1997). Inbred or near homogeneous populations of SCN HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (races 1, 2, 3 and 5, respectively) were used in this study from Dr. D.A. Sleper's lab at University of Missouri-Columbia, MO, USA. Those SCN populations have been maintained for many generations. Resistance sources 'Pickett', 'Peking', PI 88788, PI 90763, and PI 437654, PI 209332, PI 89772 were used as indicator lines to monitor the purity of HG type populations and success of the phenotyping experiments (Niblack et al., 2002). 'Hutcheson' was used as the standard susceptible control. Based on the

reaction of indicator lines to HG type populations, the experiments were performed successfully.

Eight plants were planted in the Greenhouse from each of the genotypes for each of the tested HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (races 1, 2, 3 and 5, respectively) during April 2008 for SCN bioassays. Seed of the two parents, each of the 160 $F_{2:3}$ families, the indicator and the control lines were germinated in an incubator. Individual plants of each parent and lines above were randomly transplanted into micro pots filled with steam-pasteurized Brosely fine sandy loam. Each of the plastic containers (20 cm diameter) contained twenty-five micro pots and were partially submerged in a water-bath and maintained at 27 ± 1^{0}C throughout the experiment. Three days after transplanting, each plant was inoculated with 2000±25 SCN eggs using an automatic pipetter. Thirty days after inoculation, individual plant roots were collected and washed with a strong jet of water to dislodge nematode cysts and counted under a stereomicroscope. The female index (FI) based on the standard classification system (Schmitt and Shannon, 1992) was used to evaluate SCN reaction of each individual plants including 160 $F_{2:3}$ lines, parents and indicator lines. The female index was calculated as a percentage as follows:

$$\text{FI (\%)} = \frac{\text{Mean number of females on roots in a given subfamily}}{\text{Mean number of females on roots of Hutcheson}} \times 100$$

A female index (FI) of ≤ 10% is considered a resistant (R) reaction whereas a female index (FI) >10% is defined as susceptible (S). A graphical presentation of SCN bioassay work has included in the Appendix figure 1.

Plant DNA preparation and SSR analysis

Young leaves (trifoliate) of F_2 plants from PI 567476 X SS97-6946 along with both parents were collected from BREC farm during July 2007 and stored at -80^{o}C for future use. Stored leaves were dried by using Fridge Dryer for 72 hours @ -40^{o}C and 0.133 mBars pressure. Fine powder was made from dried leaves by using automatic GenoGrinder @ 1800 strokes/minute for 3 minutes. DNA was extracted from soybean leaf powder of each F_2 individuals and parents following CTAB method (Keim et al. 1988) with minor modifications and used for SSR analysis.

Because morphological markers were identical for both parents i.e. purple flower and grey pubescence, we used SSR markers to confirm genetically, the segregation of the F_2 population among individuals before initiating next steps.

All SSR markers used in this study have been assigned to the soybean composite genetic map (Song et al., 2004). Polymerase chain reaction (PCR) was conducted in both 96 and 384 well micro plates with a final volume of 15 µl on the Eppendorf master cycler gradient (Eppendorf AG, Germany). Each reaction included 2.0 µl 10 x PCR buffer, 2.0 µl of 2µM dATP, dCTP, dTTP, dGTP, 0.1 µl of 20 µM forward and 0.15 µl of 20 µM reverse SSR primer (labeled), 1 unit Taq polymerase (Genescript Corporation, Piscataway, NJ, USA), 1.5 µl PVP (10% w/v), 1 µl of 25 mM $MgCl_2$, 25-30 ng genomic DNA and sterile water. The PCR cycling program included a first cycle at 95^{o}C for 3 min; 35 cycles at 94^{o}C for 30 sec for denaturing; 52^{o}C for 45 sec for annealing; and 72^{o}C for 1 min for extension. The reaction was terminated with a 7.0 min extension at 72^{o}C. The PCR products were run on an ABI 3100 sequencer.

A total of 547 SSR primer pairs were first screened against the two parents SS97-6946 and PI 567476 to identify polymorphic markers. The identified polymorphic primer pairs were used for genotyping the 160 F_2 individuals. A graphical presentation of genotyping work has included in the Appendix figure 2.

Statistical Analysis and Linkage Map Construction

Phenotypic data

Soybean cyst nematode reaction was determined based on number of white female development on eight plants from each $F_{2:3}$ family. $F_{2:3}$ lines were classified into two categories resistant and susceptible based on female index. A frequency distribution was determined for reaction of lines within individuals to each HG type. The Chi-square (χ^2) test using the Yates correction analysis to adjust for small population size was used to test the goodness of fit for the proposed gene models. In this study, each $F_{2:3}$ line used in different HG type or race bioassays were derived from a given F_2 individual. Hence, a single correlation coefficient analysis was used to study the relationship of host plant resistance to different SCN HG types. The Shapiro-Wilk test was conducted to measure normality of the frequency distributions and suitability for detecting QTLs for SCN resistance in the 160 F_2:$_3$ families to SCN races 1, 2, 3 and 5.

Linkage Group assignment of QTLs

The genetic linkage map was constructed using Joinmap 3.0 (Van Ooijen and Voorrips 2001). Parameters were set as default, i.e. LOD grouping thresholds $\geq$ 3.0 and a maximum distance of 50 cM. Assignment of LG was based on similarity to the integrated

soybean map (Song et al., 2004). All markers mapped to LGs were evaluated individually by the χ^2 test for goodness of fit against a 1:1 segregation ration at a 0.01 probability level.

QTL analysis

Composite interval mapping (CIM) was performed to localize and detect SCN resistance QTL using MapQTL version 5.0 (Van Ooijen and Voorrips 2001). For CIM, forward and backward stepwise regressions were performed to select 10 markers as cofactors and the analysis was conducted using model 6 with a moving window size of 10 cM. At each interval, the significance of the QTL-trait association was tested by the likelihood ratio statistics. Permutation tests were conducted 1000 times to determine a critical LOD value to minimize the experimental type I error rate. For each trait, a significant threshold level was estimated by 1000 permutations at $p< 0.05$ using the MapQTL program. However a LOD score of 3.0 was used as a threshold to declare the presence of a putative QTL. The score was employed to identify all possible regions associated with SCN resistance. The putative QTL and LG figure was created using Mapchart 2.2 program. Stepwise flow chart of QTL detection has graphically presented in Appendix figure 3.

Chapter 3

Results and Discussion

Phenotype Evaluation

Reaction of $F_{2:3}$ families with SCN

Reaction of the standard HG type indicator lines and control (Hutcheson) confirmed the purity of SCN populations (HG types) used in these studies. Results revealed that significant variation was observed for responses against all tested SCN HG types (races) among $F_{2:3}$ families, parents, controls and differentials (Table 1). This indicated that the reactions of individual plants to SCN HG types were significantly affected by environment. The frequency distribution of $F_{2:3}$ families response with SCN HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (race 1, 2, 3 and 5, respectively) showed a normal or near normal distribution (Figure 1 to 4). But the normality test revealed that SCN HG types 2.5.7 and 0 (race 1 and 3, respectively) could be normally distributed due to higher p value and SCN HG types 1.2.5.7 and 1.2.7 (race 2 and 5, respectively) were not normally distributed (Table 2). However, the effect of non-normality on QTL mapping data analysis is reported to be significantly reduced because of use of cofactor markers in composite interval mapping (Jansen et al. 1993) and permutation testing for the determination of threshold values (Yang et al., 2007, Churchill and Doerge 1994). Therefore, the data were used for further analysis without transformation. It was evident that a few $F_{2:3}$ families showed higher female index than the susceptible parent (data not presented). This could happen due to transgressive segregation among families. The

result of the reaction in all inoculations of differentials, parents and control against different SCN HG types were consistent with other reports (Lu et al., 2006; Yue et al., 2000; Chang et al., 1997; Cregan et al., 1999).

Genetics of SCN resistance

Data on segregation ratios and chi-square test for the 160 $F_{2:3}$ families evaluated against SCN HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (races 1, 2, 3 and 5, respectively) are included in Table 3. The reaction to SCN HG type 2.5.7 (race 1) showed 157 susceptible and 3 resistant $F_{2:3}$ individuals. Two resistant and 158 susceptible reaction of $F_{2:3}$ individuals were found for SCN HG types 1.2.5.7 (race 2). Reaction to HG type 0 (race 3) observed 136 susceptible and 24 resistant $F_{2:3}$ families. Seven resistant and 153 susceptible reactions to SCN HG type 1.2.7 (race 5) were obtained in tested $F_{2:3}$ families. Chi-square analysis showed that segregation ratios of susceptible and resistant families fit a three recessive gene (*rhg rhg rhg*) model for SCN HG types 2.5.7 and 1.2.5.7 (race 1 and 2, respectively) with the p value for $\chi^2 > 0.75$. Two dominant and one recessive gene model (*Rhg Rhg rhg*) was a good fit for reaction to SCN HG type 0 (race 3) with a p value for $\chi^2 > 0.73$. The proposed gene model for reaction to SCN HG type 1.2.7 (race 5), was a one dominant and two recessive gene model (*Rhg rhg rhg*) with the p value for $\chi^2 > 0.85$ (Table 3). Inheritance of SCN resistance in the $F_{2:3}$ families showed three gene models with all tested SCN HG types which are similar to several previous studies. Caldwell *et al.* (1960) first reported the inheritance of SCN resistance in Peking and reported that three recessive genes (*rhg1 rhg2 rhg3*) were involved in resistance to SCN. *Rhg4*, a dominant gene responsible for SCN resistance was additionally reported in Peking (Matson and Williams 1965). Another dominant gene *Rhg5* was identified in PI 88788 by

Arelli et al. (1992, 1994). Yue et al. (2000) found two dominant and one recessive genes (*Rhg Rhg rhg*) in PI 438489B for resistance to SCN HG type 0 (race 3) and one dominant and two recessives (*Rhg rhg rhg*) for resistance to SCN HG types 1.2.7 (race 5). In another study a three gene model (*Rhg rhg rhg*) for SCN HG types 1.2.7 (race 5) were reported for resistance (Qiu et al., 1997). Earlier studies reported that there were common genes in several PIs for different SCN HG types. A major SCN resistance gene located in the same region of LG G were found in Peking, PI 88788, PI 437654 and PI 90763, PI 438489B for HG type 0 (race 3) (Concibido et al., 2004). Several reports showed that some of these PIs had QTLs in common for resistance to HG types 2.5.7, 1.2.5.7, 0, 1.5.7 (race 1, 2, 3 and 5, respectively) (Heer et al. 1998; Webb, 2003; Yue et al., 2001a;2001b; Concibido et al., 2004).

Relationship of resistance to different SCN HG types

Phenotypic results revealed that some resistance genes might be common or are tightly linked in refining the reaction to different SCN HG types in the tested soybean populations. Soybean cyst nematode bioassay results showed that several $F_{2:3}$ families had broad-spectrum resistance to multiple races (data not shown). Two $F_{2:3}$ families showed complete resistant reaction to all tested SCN HG types 2.5.7, 1.2.5.7, 0 and 1.5.7 (race 1, 2, 3 and 5, respectively). One family had resistance to SCN HG types 2.5.7, 0 and 1.5.7 (race 1, 3 and 5, respectively) and another two families were resistant to SCN HG types 0 and 1.5.7 (race 3 and 5, respectively). Correlation analysis was conducted to evaluate the reactions to different SCN HG types for the same tested $F_{2:3}$ families. The response to all tested SCN HG types were significantly correlated (Table 4) to each other at the 0.1% level of significance. Some germplasm were shown to be resistant to multiple

races in studies conducted to evaluate responses of soybean germplasm against different SCN HG types (Young, 1995; Arelli et al., 2000). A QTL located on LG G was associated with resistant to HG types 2.5.7, 0 and 1.7 (races 1, 3 and 6, respectively) (Concibido et al., 1996). Previous studies reported that the responses to different SCN populations were highly correlated (Yue et al., 2000; Lu et al., 2006). Some common molecular markers in different linkage groups were closely linked and responsible for resistance to multiple SCN HG types (Yue et al., 2001a, 2001b, Guo et al. 2006a). Inheritance of SCN resistance in SS97-6946 is complex like inheritance in other resistance sources have shown and could contain major genes for resistance.

DNA Marker Analysis and Mapping

Confirm Segregation among the population

Twenty F_2 plants were randomly chosen along with parents to confirm segregation among populations. Ten primer pairs were used to analyze genetically selected materials. Out of ten markers, four showed clear polymorphism among individuals (data not showed). This confirmed that there was segregation among F_2 individuals derived from the PI 567476 x SS97-6946 cross.

Polymorphism Survey

Three hundred forty-seven out of 547 (<63%) SSR primer pairs were found polymorphic between PI 567476 and SS97-6946. These 347 polymorphic primer pairs were selected covering all twenty LGs of soybean to screen 160 $F_{2:3}$ families derived from the cross PI 567476 x SS97-6946.

Genetic linkage map construction

All selected polymorphic primer pairs (total 347) were used in SSR analysis. Three-hundred forty-seven polymorphic primer pairs were nearly distributed among the lengths of the 20 LGs in the soybean composite linkage map (Song et al., 2004). Among 347 markers, 20 markers were discarded from further analysis because of missing data or did not amplify. Due to severe deviation from the expected segregation ratios, a total of 10 markers were not used for genetic mapping. Twenty-two SSR markers were observed to be dominant leaving 295 markers to possibly be placed on the linkage map. The software Joinmap 3.0 (Van Ooijen and Voorrips 2001) was used to construct LG using 295 good markers.

Finally, 263 markers were positioned on the linkage map, which comprised 39 LGs. Compared to the soybean composite map (Song et al., 2004); the linkage map constructed with the 160 F_2's was very consistent in marker arrangement and relative distance between SSR markers with exception of a few regions (Figure 5). Eighteen markers were not assigned to the same order as the soybean composite map (Song et al., 2004) and these were not used. Fourteen markers were unassigned and not included. The linkage map spanning a total of 2550.1 cM across 39 LGs comprised 101% of the integrated soybean map (Song et al., 2004) and 99.99% of the soybean genetic map reported by Choi et al., (2006). The highest number of markers (26) was placed in LG G followed by LG O (18). Single gaps (≥50 cM between neighboring markers) occurred in LGs A2, B2, C1, C2, D1a, D2, E, J, K, L and M (chromosome no. 8, 14, 4, 6, 1, 17, 15, 16, 9, 19 and 7), respectively. Therefore eleven subgroups were formed, one in each respective LG in order according to the soybean composite map. Double gaps (≥50 cM between

neighboring markers) existed in LG I (chromosome no. 20) according to soybean composite map. Two subgroups were formed in LG I. Triple gaps (≥50 cM between neighboring markers) existed in LGs D1b and F (chromosome no. 2 and 13), respectively. Three subgroups were formed each in LGs D1b and F in order according to soybean composite map.

Markers assigned were uniformly disseminated along LGs except a few big gaps such as 45.7 cM gap between Sat_417 and Satt656 on LG F subgroup; 42.7 and 38.8 cM gap between Sat_158 - Satt142 and Sat_122 - Sat_158, respectively on LG H; 41.2 cM gap between Sat_289 and Satt271 on LG D1b subgroup. This occurred even though large numbers of SSR markers were screened for these LGs. This incomplete coverage reflected the low level of polymorphism in these gap regions between the parents.

QTLs Conditioning SCN Resistance

Results revealed that SCN resistance is controlled by multiple, largely diverse loci that are distributed throughout the genome. Individual QTLs for SCN resistance accounted for 3.1 to 27.7 % of the total phenotypic variation (Table 5). A LOD score of 3.0 was considered to be the threshold for declaring presence of a suggestive QTL, but permutation tests (with 1000 permutations at ($p<0.05$) run by MapQTL to determine a critical LOD value for minimizing the experimental type I error rate. Significant threshold LOD scores ($p<0.05$) were obtained (3.8 to 4.2 with an average of 4.05). Almost all previous reported studies used a LOD score 3.0 to declare their QTL as suggestive (Guo et al. 2005; 2006a; Yue et al. 2001a; 2001b; Concibido et al., 1996; Webb et al., 1995; Heer et al., 1998; Qiu et al., 1999; Wang et al., 2001; Meksem et al.,

2001). We chose a less stringent LOD score (3.0) to maximize the likelihood of identifying map locations associated with SCN resistance because the population size (160 $F_{2:3}$ families) is somewhat small and to keep consistent with earlier studies.

HG type 2.5.7 (race 1)

Three putative QTL associated with resistance to SCN HG type 2.5.7 (race 1) were detected and placed on LGs A2, E and G (Fig 5 and Table 5). Among these identified QTL, the QTL located between SSR marker Sat_315 and Sat_403 on LG G (chromosome no. 18) explained 18.8% of the total phenotypic variation with strong statistical support (LOD Value=6.95). Here the marker Satt610 was linked with detected QTL. According to soybean composite map, Satt610 is placed 6.3 cM downstream of Marker Satt309, which was tightly linked with SCN resistance gene *rhg1* (Cregan et al., 1999a, b; Meksem et al., 2001). Therefore, SS97-6946 may contain SCN resistance gene *rhg1*. QTL on LG A2 (chromosome no. 8) located between marker Satt315 and Sat_157 with 5.3 cM interval and contributed 7.8% of the total phenotypic variation (Table 5). Earlier studies showed that the gene *Rhg4* was mapped in the region of Satt632-pBlt65-I and associated with resistance to SCN HG types 2.5.7 and 0 (races 1 and 3) (Meksem et al., 2001; Cregan et al., 1999b; Heer et al., 1998). According to soybean composite map (Song et al., 2004) the marker interval identified in our study overlaps with Satt632-pBlt65-I. Thus, it is likely that the detected QTL carries *Rhg4* for SCN HG type 2.5.7 (race 1) resistance. The QTL located on LG E (chromosome no. 15) were assigned between markers Sat_107 and Satt483 with 7.8 cM distance and explained 7.2% of the total phenotypic variance. So far no QTL resistant to SCN HG type 2.5.7 (race 1) on LG E has been reported in any previous study but QTL resistant to SCN HG type 1.2.5.7, 0

and 1.2.7 (race 2, 3 and 5, respectively) on LG E were reported by others (Yue et al., 2001a; 2001b; Wang et al., 2001; Guo et al., 2006a) in the same region of the soybean composite map. SS97-6946 was the possible source of resistance gene of QTL identified on LGs A2 and G and resistance gene of QTL on LG E could be come from PI 567476 (Table 5).

HG type 1.2.5.7 (race 2)

Only one putative QTL associated with resistance to SCN HG type 1.2.5.7 (race 2) was indentified and located between SSR markers Satt684 and Satt382 on LGs A1 (chromosome no. 5) and explained 18.8% of the total phenotypic variation with strong supporting statistical evidence a LOD Value = 5.19 (Fig 5 and Table 5). SS97-6946 worked as source of resistance gene of this QTL (Table 5). Yue et al. (2001a) identified QTL associated with resistance to SCN race 2 in LG A1 in PI439489B that was close to the same interval. According to soybean composite map the end of the marker interval identified in our study is 4.5 cM upstream from the beginning of the marker interval found by Yue et al. (2001a) for QTL resistance to SCN race 2 in LG A1 in PI439489B. But Vierling et al. (1996) reported QTL resistant to SCN race 3 in the same region as reported here.

HG type 0 (race 3)

Three QTL associated with resistance to SCN HG type 0 (race 3) were assigned on LG A2, G and M (Fig 5 and Table 5). The QTL on LG G (chromosome no. 18) provided the highest portion (9.5%) of the total phenotypic variation for SCN resistance to race 3 (Table 5) and was located between markers Sat_315 and Sat_403. Satt610 was placed as

linked marker with detected QTL in this case. According to soybean composite map marker Satt610 is slightly located 6.3 cM downstream of Marker Satt309, which was tightly linked with SCN resistance gene *rhg1* (Cregan et al., 1999a, b; Meksem et al., 2001; Concibido et al., 2004; Guo et al., 2005; 2006a). QTL identified in our study fell in the same region as ones identified by other studies on LG G, resistant to SCN race 1 and 3 in PI 209332, and Peking (Concibido et al., 1994 and Heer et al., 1998). Therefore, soybean line SS97-6946 could contain SCN resistance gene *rhg1*. The other QTL detected on LG A2 (chromosome no. 8) contributed 9.2% of the total phenotypic variation effects for SCN resistance to race 3 (Table 5) and was flanked by markers Satt315 and Sat_157 in a 5.3 cM region with a significant LOD value of 4.63. Earlier studies showed that *Rhg4* located at markers Satt632-pBlt65-I was mapped, and was shown to be resistant to SCN HG types 2.5.7 and 0 (races 1 and 3) (Meksem et al., 2001; Cregan et al., 1999b; Heer et al., 1998). According to soybean composite map (Song et al., 2004) our marker interval overlaps with Satt632-pBlt65-I. Thus, it is likely that the detected QTL carries *Rhg4* for SCN HG type 0 (race 3) resistance. QTL detected on LG M (chromosome no. 7) resistant for SCN HG type 0 (race 3) were flanked with markers Sat_121 and Satt551 in a 10.0 cM region. Heer et al. (1998) and Webb et al. (1995 and 2003), with single marker analysis, assigned minor QTLs linked with markers A131 and php020301a, respectively on LG M associated with SCN HG types 2.5.7, 0 and 1.2.7 (races 1, 3 and 5, respectively) resistances. The interval associated with HG type 0 (race 3) resistance detected in this study was distant from markers reported in J87-233 and PI 437654. Thus, the reported soybean accessions may not contribute to the common

resistance genes with SS97-6946 on LG M to race 3. SS97-6946 was the only resistance source for all of those identified QTL associated with SCN race 3 (Table 5).

HG type 1.2.7 (race 5)

The highest number of resistance QTL (8 total) were detected against SCN HG type 1.2.7 (race 5) on soybean LGs A1, B1, B2, G, M and O (Figure 5 and Table 5). One putative QTL associated with resistance to SCN HG type 1.2.7 (race 5) was indentified and located between SSR markers Satt684 and Satt382 on LGs A1 (chromosome no. 5) and explained 27.7% of the total phenotypic variation (LOD Value=13.15) (Fig 5 and Table 5). Yue et al. (2001a) identified QTL associated with resistance to SCN race 2 on LG A1 in PI439489B that is close to but different than the interval identified in this study. According to soybean composite map, the end of the marker interval identified in this study is 4.5 cM upstream from the beginning of the marker interval found by Yue et al. (2001a) for QTL associated with resistance to SCN race 2 in LG A1 in PI439489B. But Vierling et al. (1996) reported QTL resistant to another SCN race 3 in the same region as we found in our study. This QTL region could be a new resistance source against SCN HG type 1.2.7 (race 5). Three LOD peaks associated with resistance QTL with SCN race 5 were observed on LG G (chromosome no. 18). The highest LOD (10.22) peaks were associated with marker intervals Sct_199 and Satt191 witch shared 12.9% of the total phenotypic variation, followed by a LOD value 4.05 associated with marker intervals Sat_131 and Sat_315 which shared 11.8% of the total phenotypic variation. The lowest LOD value, 3.65 was associated with marker intervals Sat_315 and Sat_403 which explained 5.8% of the total phenotypic variation. No QTL has been reported for resistance to SCN race 5 in the region of marker interval Sct_199 and Satt191; however,

resistance for SCN race 3 was mapped in the same region (Wang et al., 2001 and Yue et al., 2001b). The other two QTL mapped in LG G in our study could be the same QTL due to markers associated with QTLs located in the same region in the soybean composite map (Song et al., 2004) and identified as associated with SCN resistance to multiple races. QTL associated with resistance to SCN HG type 1.2.7 (race 5) mapped on LG B1 (chromosome no. 11) and located between makers Satt444 – Satt665 explained 5.7% of the total phenotypic variation but LOD value (2.95) was little bit lower than suggestive value (Table 5). Yue et al. (2001a) mapped resistance QTL for SCN races 1, 2 and 5 on LG B1 in soybean PI 438489B which overlapped the QTL region identified in this study. In other studies the QTL region mapped close to the QTL resistance for race 5 on LG B1 in this study (Guo et al., 2006a and Webb, 2003). Thus, QTL identified in the tested accession could be the same as previously detected in LG B1. QTL on LGs B2, M and O (chromosome no. 14, 7 and 10, respectively) flanked with markers Sat_287 – Satt126, Sat_147 – Satt551 and Satt173 – Sat_282 explained 5.1%, 4.4% and 3.1%, respectively of the phenotypic effect for resistance to SCN race 5. No QTL associated with resistance to SCN race 5 has been reported on LG B2. Two QTL for resistance to race 1 and race, however, mapped to a different region on LG B2 (Qui et al., 1999 and Yue et al., 2001a). The interval associated with resistance to HG types 2.5.7, 0 and 1.2.7 (races 1, 3 and 5, respectively) detected in our study was distant from minor QTLs linked with markers A131 and php020301a on LG M reported in J87-233 and PI 437654 (Heer et al., 1998 and Webb et al., 1995 & 2003). Thus, genes from these soybean accessions may not be in common with the genes from SS97-6946 on LG M for Race 5 resistance. Very recently one QTL associated with resistance to SCN race 2 was identified but not confirmed on

LG O with a minimum LOD value 2.1 which matched the resistance QTL to race 5 in this study (Winter et al., 2007a). Therefore, QTL identified on LGs B2, M and O could be a novel resistance locus for SCN race 5. The resistance gene of QTL on LGs A1, M, O and two QTLs (out of three) on LG G came from SS97-6946 but rest of the QTL's resistance gene could be come from PI 567476 (Table 5).

QTLs for SCN resistance for different races were classified into three categories including confirmed QTLs, suggestive QTLs and significant QTLs (Guo et al., 2006b). QTL associated with SCN resistance have been confirmed on LGs A2, B1, E, G and J (Concibido et al., 2004, Guo et al., 2006b), but only QTLs on G (*rhg1*) and A2 (*Rhg4*) are reported to be cloned and sequenced (Meskem et al., 2001; Ruben et al., 2006). In this study, QTLs on LGs A2, G, E and B1 were also associated with resistance to HG types 2.5.7, 0, and 1.2.7 (races 1, 3 and 5, respectively). The QTL in the same region on LG G is likely to be *rhg1* and was associated with resistance to SCN HG types 2.5.7, 0, and 1.2.7 (races 1, 3 and 5, respectively). The QTL on LG E was located in the same region as reported in other mapping studies (Concibido et al., 2004, Guo et al., 2006b) and was associated with SCN resistance to HG type 2.5.7 (race 1), but not with HG type 0, and 1.2.7 (races 3 and 5, respectively). The QTL in the same region on LG A2 was associated with SCN resistance to HG types 2.5.7 and 0 (races 1 and 3, respectively) but not with HG type 1.2.7 (race 5) (Concibido et al., 2004, Guo et al., 2006b). The QTL located in the same region on LG B1 as reported in other mapping studies (Concibido et al., 2004, Guo et al., 2006b) was associated with SCN resistance to HG type 1.2.7 (race5) but not with HG type 2.5.7 and 0 (races 1 and 3, respectively). These results are consistent with previous reports (Guo et al., 2006b). However, QTL on LGs A1, M and O

associated with HG types 1.2.5.7, 0, and 1.2.7 (races 2, 3 and 5, respectively) in SS97-6946, have not been reported, and could be a novel putative locus for SCN resistance for respective races. Another possible novel QTL has identified on LG B2 but the resistance gene associated with HG type 1.2.7 (SCN race 5) could be come from other parent. A confirmation study is needed to verify these results.

Chapter 4

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Tables and Figures

Table 1: Mean and range of Female Index of $F_{2:3}$ lines from population PI567476 x SS97-6946 and parents to SCN races

Lines	Race 1		Race 2		Race 3		Race 5	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
SS97-6946	0.4	0.0-1.4	4.3	0.0-8.0	0.1	0.0-0.7	0.0	0.0-0.0
PI 567476	35.4	28 –46.4	47.6	21.3-85.8	21.8	14.7-29.5	20.6	9.7-32.7
Pop 6946 ($F_{2:3}$)	36.1	0.9–70.9	36.8	9.1 –78.4	21.3	0.2 -50.2	27.5	1.3-61.6
Hutcheson*	211	191-231	187.5	155-208	135.8	78-174	143.8	122.0-180.0

* Assign mean no. of female (cyst).

Table 2: Normality test of female index for $F_{2:3}$ individuals from PI 567476 x SS97-6946 to SCN races 1, 2, 3 and 5

Race	Sharpiro-Wilk's	p value	Skewness	Kurtosis
Race 1	0.987837	0.1798	-0.169683	0.87265456
Race 2	0.967722	0.0009	0.7039851	0.85536947
Race 3	0.985365	0.0906	0.38395221	-0.0203205
Race 5	0.953866	0.0001	0.79359965	0.45650631

Table 3: Genetic analysis and reaction of $F_{2:3}$ individuals of PI 567476 x SS97-6946 to SCN races 1, 2, 3 and 5

Races	No. of Plants			Hypothesized resistance genes	Expected genetic ratio	χ^2	p-value
	Total	Observed ratio R:S	Expected ratio R:S				
Race 1	160	3:157	2.5:157.5	rhg rhg rhg	1:63	0.102	0.750
Race 2	160	2:158	2.5:157.5	rhg rhg rhg	1:63	0.102	0.750
Race 3	160	24:136	22.5:137.5	Rhg Rhg rhg	9:55	0.116	0.733
Race 5	160	7:153	7.5:152.5	Rhg rhg rhg	3:63	0.035	0.852

Table 4: Correlation coefficients of reaction of $F_{2:3}$ individuals to the different SCN HG types (races).

	Race 1	Race 2	Race 3	Race 5
Race 1	1.00			
Race 2	0.367**	1.00		
Race 3	0.547**	0.252**	1.00	
Race 5	0.511**	0.365**	0.271**	1.00

** p value < 0.001

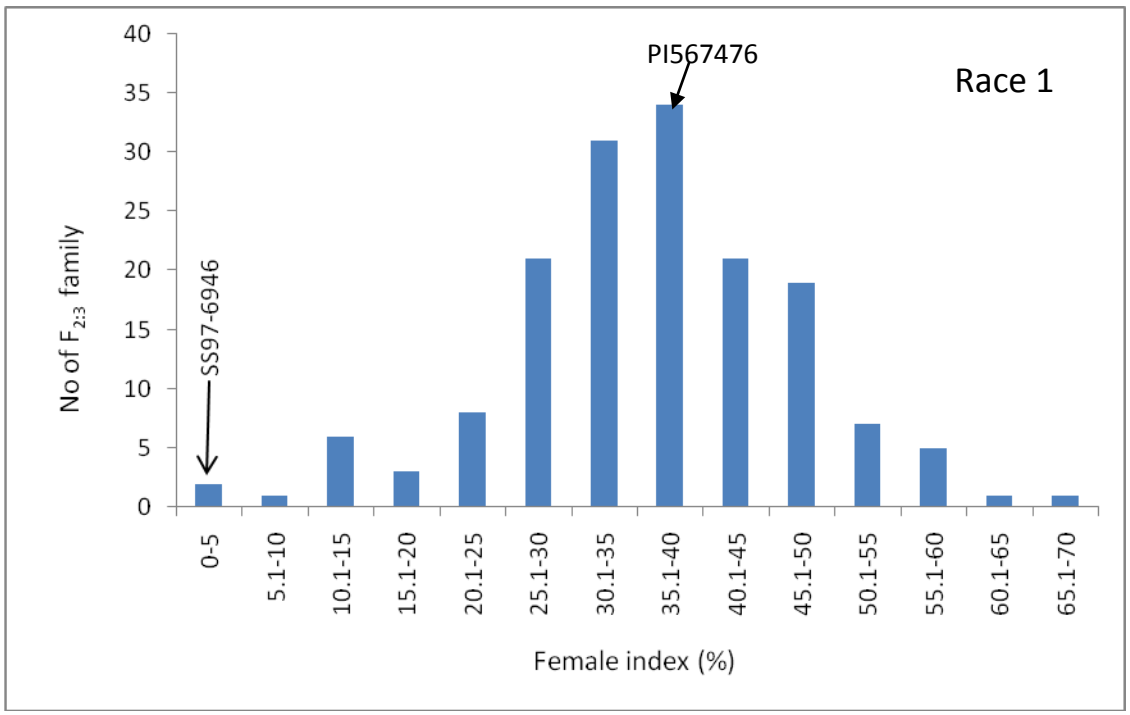


Figure 1. Frequency distribution for female index (FI) to SCN HG types 2.5.7 (race 1)

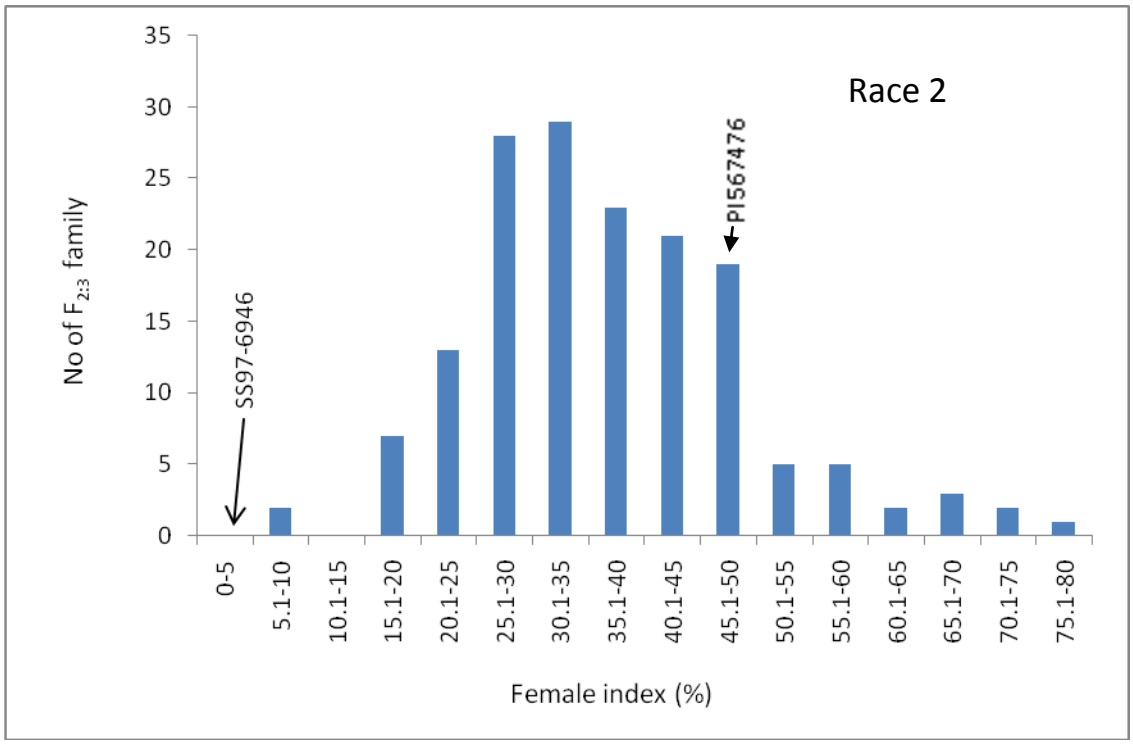


Figure 2. Frequency distribution for female index (FI) to SCN HG types 1.2.5.7 (race 2)

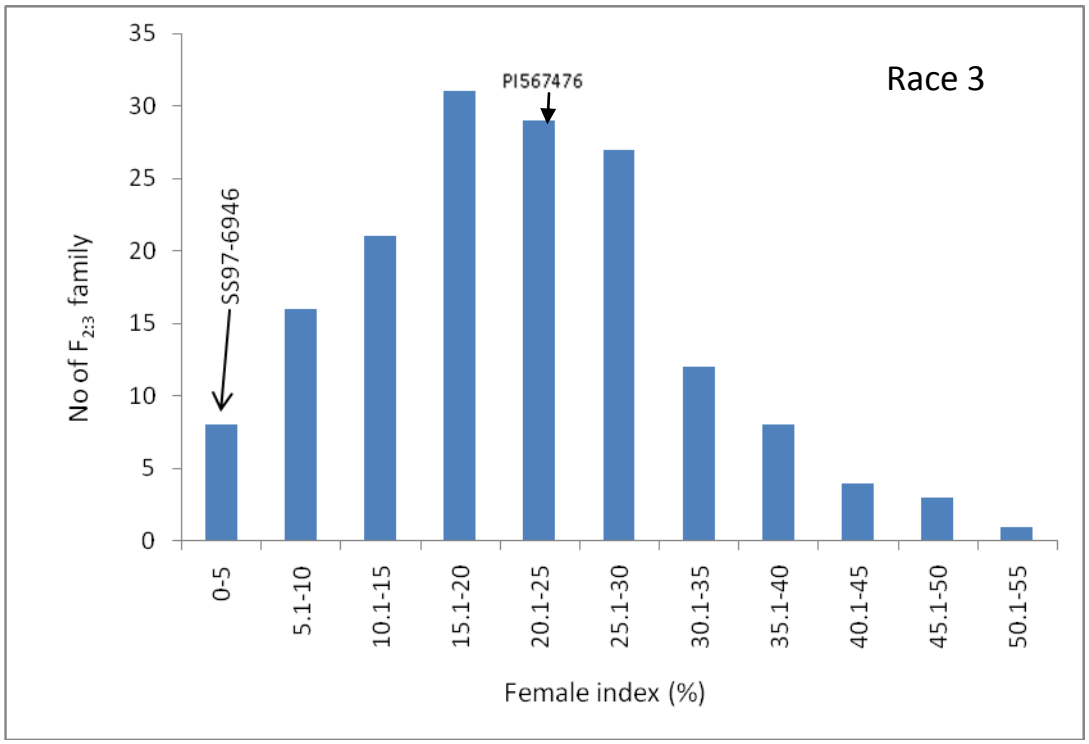


Figure 3. Frequency distribution for female index (FI) to SCN HG types 0 (race 3)

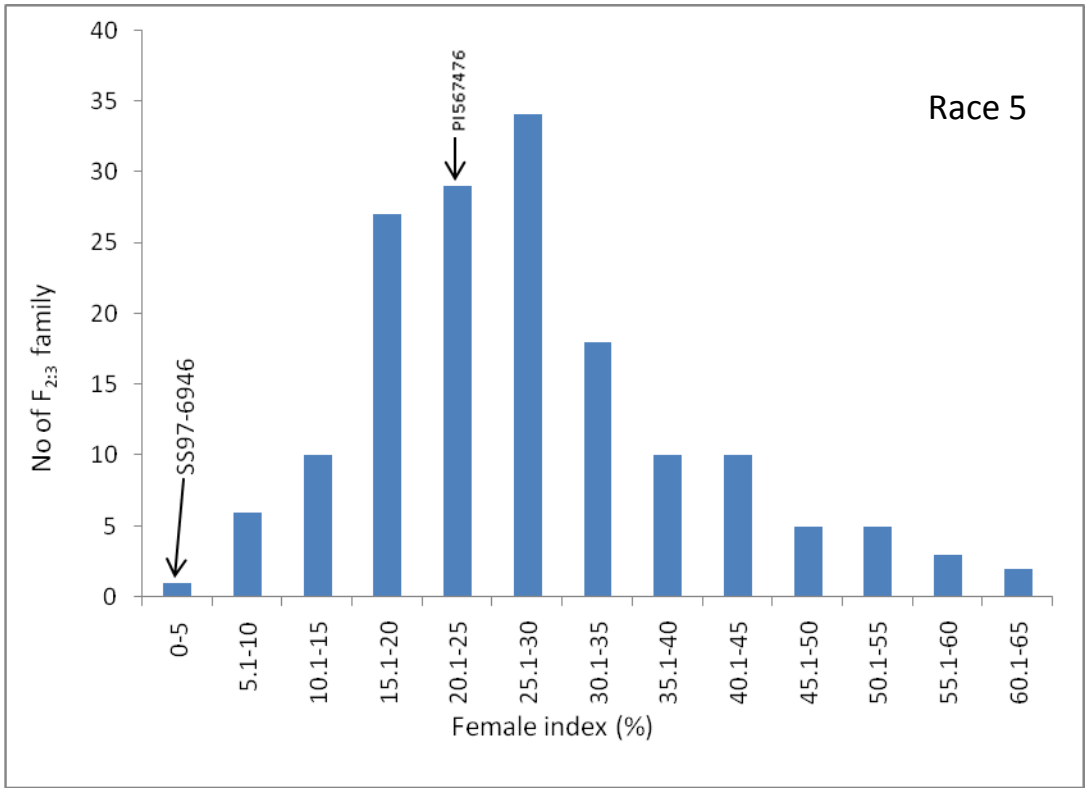


Figure 4. Frequency distribution for female index (FI) to SCN HG types 1.2.7 (race 5)

Table 5: QTLs associated with broad spectrum SCN resistance detected through composite interval mapping (CIM) in tested population

SCN race	LG[a]	Chr. #	Marker interval	Linked marker[b]	Length (cM)[c]	QTL position[d]	LOD	R^2 (%)[e]	Cum. R^2 (%)	Possible source of resistance gene
1	A2	8	Satt315-Sat_157	SUIC100-8K	5.3	4.0	3.16	7.8	29.1	SS97-6946
	E	15	Sat_107-Satt483	Satt268	7.8	4.0	3.25	7.2		PI 567476
	G	18	Sat_315-Sat_403	Satt610	23.5	12.0	6.95	18.8		SS97-6946
2	A1	5	Satt684-Satt382	Sat_368	30.9	20.0	5.19	18.8	18.8	SS97-6946
3	A2	8	Satt315-Sat_157	SUIC100-8K	5.3	5.3	4.63	9.2	30.5	SS97-6946
	G	18	Sat_315-Sat_403	Satt610	27.5	21.1	3.12	9.5		SS97-6946
	M	7	Sat_121-Satt551	Satt551	10.0	6.0	3.15	6.2		SS97-6946
5	A1	5	Satt684-Satt382	Sat_368	21.9	14.9	13.15	27.7	58.9	SS97-6946
	B1	11	Satt444-Satt665	Satt665	6.78	6.7	2.95	5.7		PI 567476
	B2	14	Sat_287-Satt126	Sat_287	13.0	5.0	3.71	5.1		PI 567476
	G	18	Sct_199-Satt191	Sct_187	16.6	11.6	10.22	12.9		SS97-6946
	G	18	Sat_131-Sat_315	Satt594	4.2	1.0	4.05	11.8		PI 567476
	G	18	Sat_315-Sat_403	Satt610	20.5	17.5	3.65	5.8		SS97-6946
	M	7	Sat_147-Satt551	Satt551	22.9	8.0	3.41	4.4		SS97-6946
	O	10	Satt173-Sat_282	Satt173	5.7	0.2	3.01	3.1		SS97-6946

a Linkage group

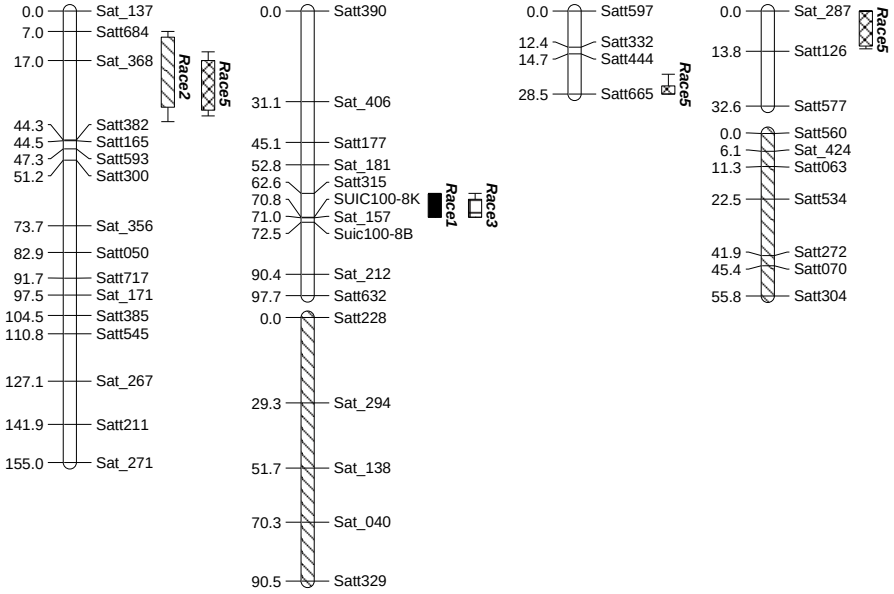
b Marker used as cofactor in CIM

b Distance of the QTL on the LG given in centiMorgans from Join Map and MapQTL

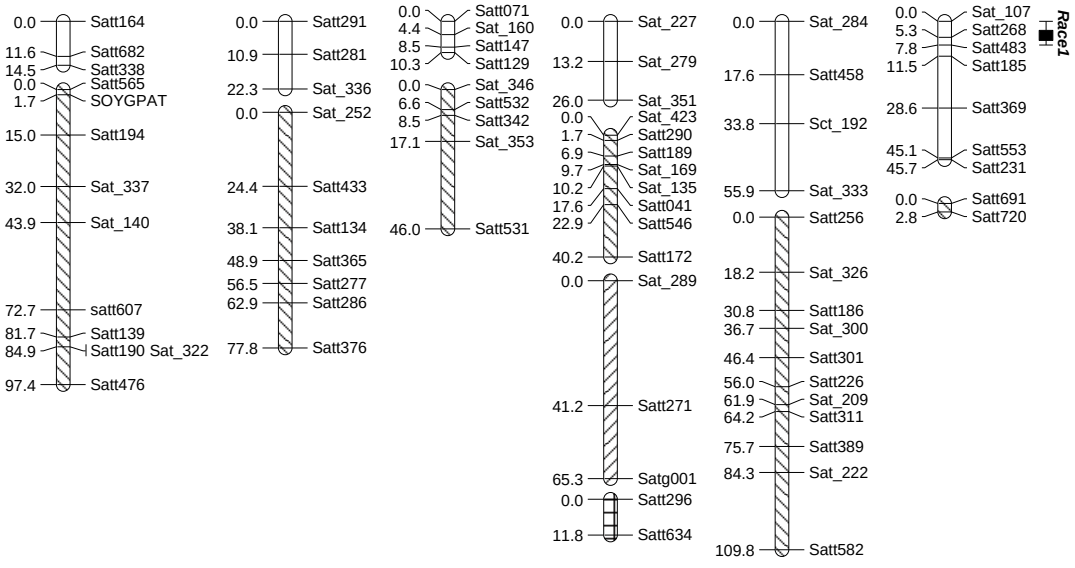
c QTL position expressed as the distance from the first marker

e Production of total phenotypic variation shared by QTL

A1(Chr.#1) **A2(Chr.#8)** **B1(Chr.#11)** **B2(Chr.#14)**



C1(Chr.#4) **C2(Chr.#6)** **D1a(Chr.#1)** **D1b(Chr.#2)** **D2(Chr.#17)** **E(Chr.#15)**



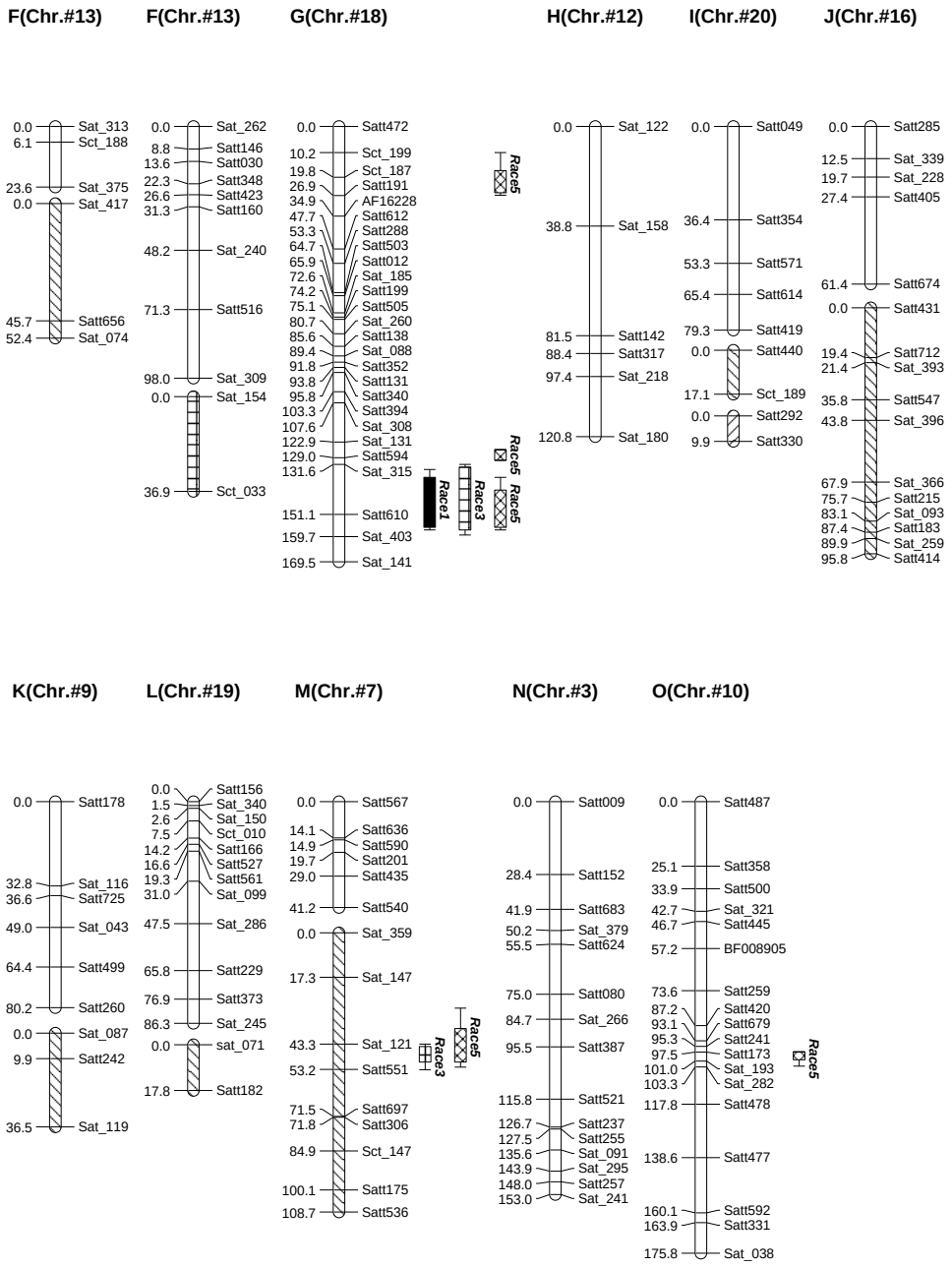


Figure 5: Linkage groups and QTL locations associated with SCN resistance to HG types 2.5.7, 1.2.5.7, 0 and 1.2.7 (race 1, 2, 3 and 5, respectively) in tested population. Linkage was declared at LOD $\geq$ 3.0 with a maximum distance of 50 cM. Detected QTL are designated by the bars with respective race designation on the right of the LG. The open bar of the LG represents the main group and crossed bars represent the sub group of the respective LGs (in the parentheses followed by linkage group denote chromosome no.).

Appendices

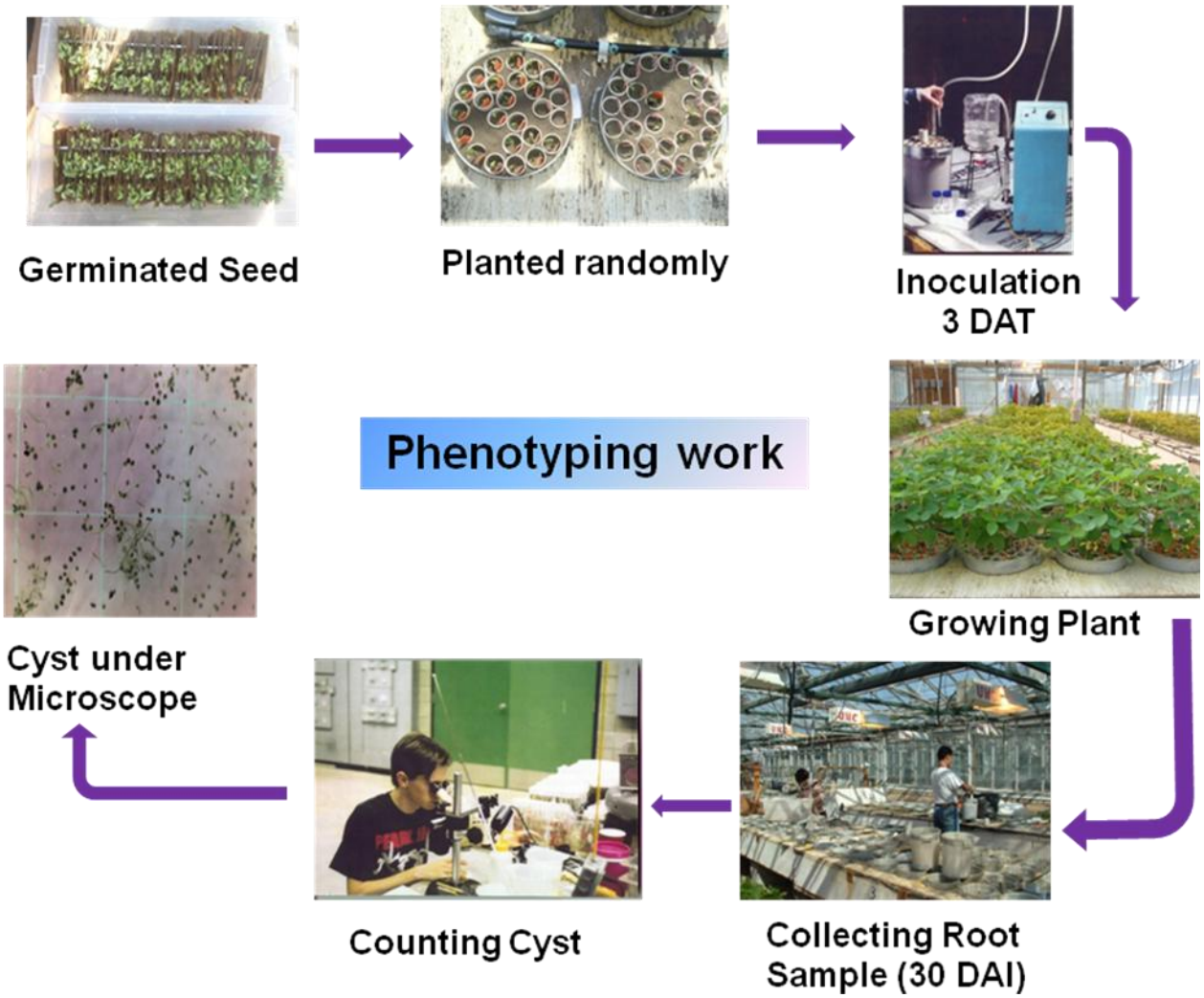


Fig 1. Flow chart of SCN bioassay in green house

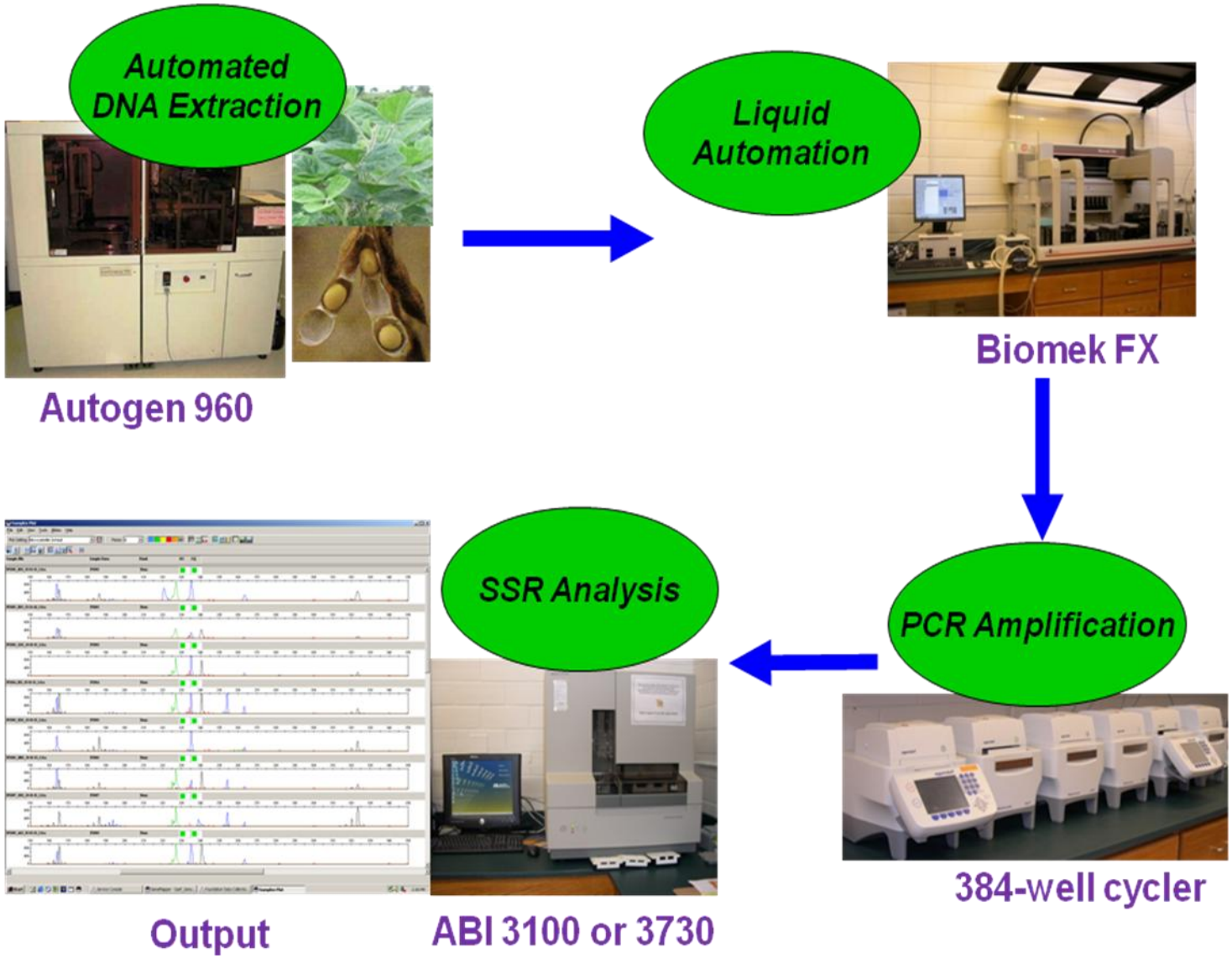
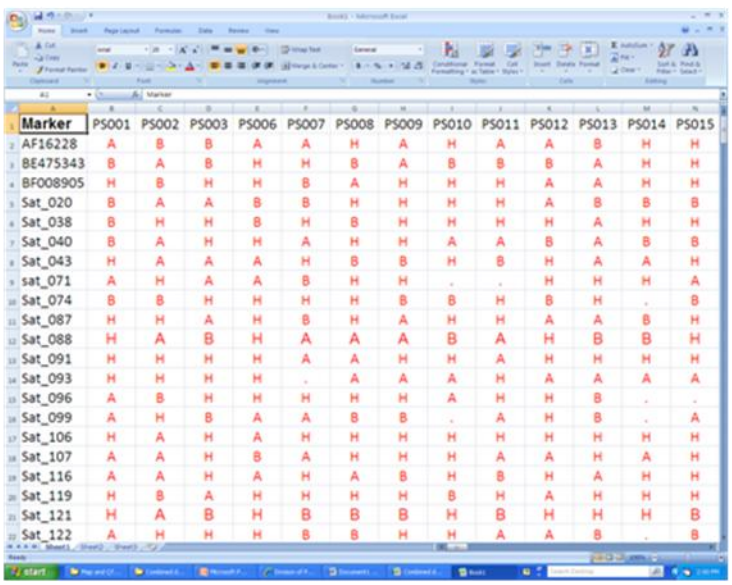
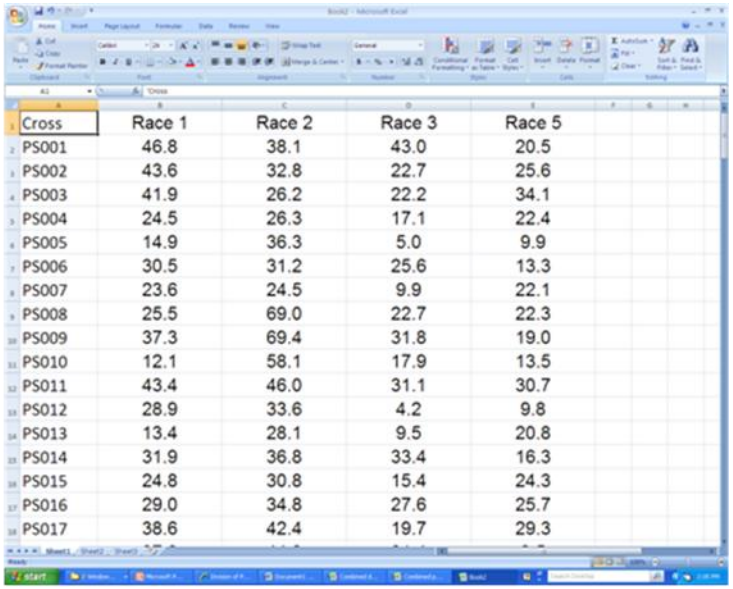


Fig 2. Flow chart of Genotyping work



Marker	PS001	PS002	PS003	PS006	PS007	PS008	PS009	PS010	PS011	PS012	PS013	PS014	PS015
AF16228	A	B	B	A	A	H	A	H	A	A	B	H	H
BE475343	B	A	B	H	H	B	A	B	B	B	A	H	H
BF008905	H	B	H	H	B	A	H	H	H	A	A	H	H
Sat_020	B	A	A	B	B	H	H	H	H	A	B	B	B
Sat_038	B	H	H	B	H	B	H	H	H	H	A	H	H
Sat_040	B	A	H	H	A	H	H	A	A	B	A	B	B
Sat_043	H	A	A	A	H	B	B	H	B	H	A	A	H
sat_071	A	H	A	A	B	H	H	.	.	H	H	H	A
Sat_074	B	B	H	H	H	H	B	B	H	B	H	.	B
Sat_087	H	H	A	H	B	H	A	H	H	A	A	B	H
Sat_088	H	A	B	H	A	A	A	B	A	H	B	B	H
Sat_091	H	H	H	H	A	A	H	H	A	H	H	H	H
Sat_093	H	H	H	H	.	A	A	A	H	A	A	A	A
Sat_096	A	B	H	H	H	H	H	A	H	H	B	.	.
Sat_099	A	H	B	A	A	B	B	.	A	H	B	.	A
Sat_106	H	A	H	A	H	H	H	H	H	H	H	H	H
Sat_107	A	A	H	B	A	H	H	H	A	A	H	A	H
Sat_116	A	A	H	A	H	A	B	H	B	H	A	H	H
Sat_119	H	B	A	H	H	H	H	B	H	A	H	H	H
Sat_121	H	A	B	H	B	B	B	H	B	H	H	H	B
Sat_122	A	H	H	H	B	B	H	H	A	A	B	.	B



Cross	Race 1	Race 2	Race 3	Race 5
PS001	46.8	38.1	43.0	20.5
PS002	43.6	32.8	22.7	25.6
PS003	41.9	26.2	22.2	34.1
PS004	24.5	26.3	17.1	22.4
PS005	14.9	36.3	5.0	9.9
PS006	30.5	31.2	25.6	13.3
PS007	23.6	24.5	9.9	22.1
PS008	25.5	69.0	22.7	22.3
PS009	37.3	69.4	31.8	19.0
PS010	12.1	58.1	17.9	13.5
PS011	43.4	46.0	31.1	30.7
PS012	28.9	33.6	4.2	9.8
PS013	13.4	28.1	9.5	20.8
PS014	31.9	36.8	33.4	16.3
PS015	24.8	30.8	15.4	24.3
PS016	29.0	34.8	27.6	25.7
PS017	38.6	42.4	19.7	29.3

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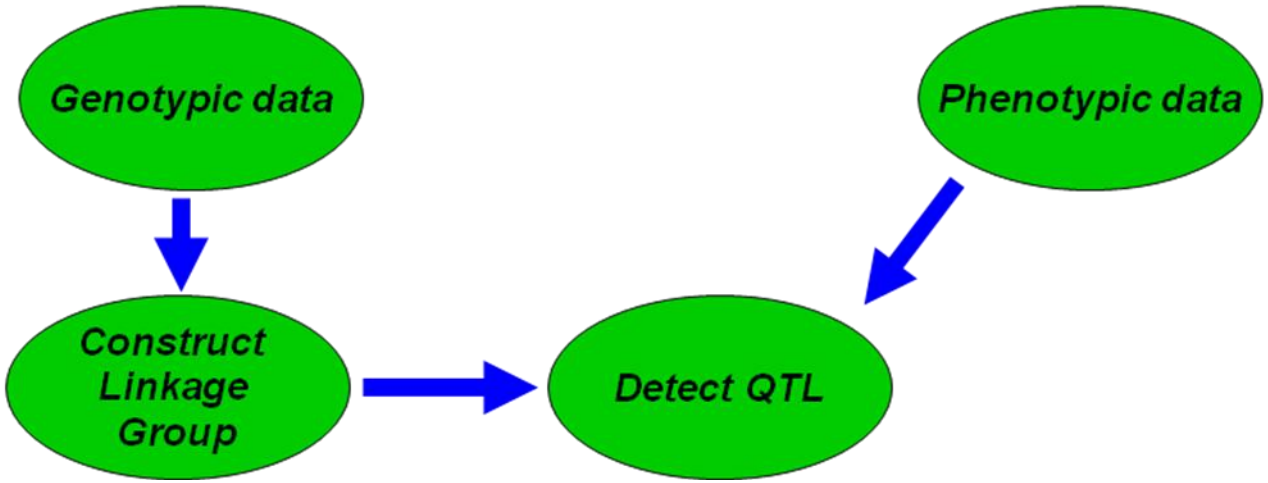

Fig 3. Flow chart of QTL detection from raw data.