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INHERITANCE OF RESISTANCE AND MOLECULAR MAPPING OF SOYBEAN APHID RESISTANCE GENES IN SOYBEAN PI 567585A; IDENTIFICATION OF APHID RESISTANCE GENES IN SOYBEAN USING MODIFIED NESTED ASSOCIATION MAPPING (MNAM)

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Menghan Liu

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By

Menghan Liu

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ABSTRACT

INHERITANCE OF RESISTANCE AND MOLECULAR MAPPING OF SOYBEAN APHID RESISTANCE GENES IN SOYBEAN PI 567585A; IDENTIFICATION OF APHID RESISTANCE GENES IN SOYBEAN USING MODIFIED NESTED ASSOCIATION MAPPING (MNAM)

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The soybean aphid, *Aphis glycine* (Matsumura), is a new major pest of soybean in the Midwest, including Michigan. Soybean PI 567585A has strong resistance to soybean aphids. The inheritance of aphid resistance in PI 567585A was determined by crossing with two susceptible soybean cultivars, Skylla and IA2070. The parents, F_1, F_2 plants and $F_{2:3}$ families were evaluated for aphid resistance in the field during the summer of 2008 and 2009. All F_1 plants exhibited intermediate phenotypes to soybean aphids. The observed segregation ratios in the two F_2 populations, and $F_{2:3}$ families fitted a segregation ratio 1:2:1. These data indicated that the aphid resistance in PI 567585A was controlled by one major co-dominant gene. Then, the genetic basis of aphid resistance in PI 567585A was determined. A mapping population of 158 $F_{4:5}$ recombinant inbred lines (RILs) derived from the cross between PI 567585A and 'Skylla' was evaluated for aphid resistance in both greenhouse and field in 2009. A single aphid resistance gene was

mapped in an interval between Satt674 and Sct_065 on linkage group J) using the composite interval mapping method. The locus explained 93.1% of the phenotypic variation in the field trial, and is located in the same genomic region as *Rag3*. This single aphid resistance gene in PI 567585A was confirmed in another $F_{3:4}$ RIL population derived from a cross between PI 567585A and IA2070.

PI 567598B was found to possess antibiosis resistance to the soybean aphid. A modified nested association mapping (MNAM) approach was used to locate resistance genes in PI 567598B on the integrated soybean linkage map. PI 567598B was crossed with 10 different susceptible cultivars to construct 10 recombinant inbred lines (RILs) populations. Genomic regions on linkage groups F, G, J and M were found associated with soybean aphid resistance in MNAM. Linkage analysis of a population of 94 $BC_1F_{4:5}$ RILs derived from PI 567598B and a $F_{4:5}$ RIL population derived from E06902 were used to confirm the MNAM results. The results of linkage analysis showed that genomic regions on the linkage groups J, F and N were associated with aphid resistance.

DEDICATION

To my family, who has supported so much to bring me to where I am today.

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INTRODUCTION AND GENERAL OBJECTIVES

The cultivated soybean, *Glycine max* (L.) Merrill. (2n=2x=40), originated in eastern Asia, and was first introduced to the United States as a forage crop in the late 1700s. Since the 1940s, soybean has become the second most important crop in the U.S. after corn. In 2009, the area planted to soybean was 31.36 million ha, and soybean production is predicted at 91.45 million tons. Numerous species of insect pests feed on soybeans, some of which can cause yield loss and even crop failure. The soybean aphid, *Aphis glycines* (Matsumura) is a new major pest of soybean in the Midwest, including Michigan. This insect, native to eastern and southern Asia, has caused significant damage throughout the soybean growing areas of Michigan and the surrounding states since 2001.

As a component of integrated pest management (IPM) strategy, host plant resistance has been recognized as an effective and environment-friendly approach to controlling soybean aphid. Several aphid-resistant soybean accessions have been identified from soybean germplasm since 2004. Then the inheritance pattern of major resistance genes in these accessions has been determined by classical genetic studies. Linkage mapping and association mapping are two widely used methods to localize the resistance genes in crop plants. To date, only linkage mapping has been used to discover aphid resistance genes in several soybean accessions. Moreover, nested association mapping (NAM) has recently been proposed to map gene(s) accurately and efficiently, by combining the advantages of linkage mapping and association mapping.

In this dissertation, the general objective is to identify molecular markers closely linked to the aphid resistance genes in two accessions, PI 567585A and PI 567598B, providing breeders with various options to exploit the pyramiding of resistance genes in cultivar development. PI 567585A is a new aphid resistance source, but the inheritance pattern is still unknown, and genetic mapping of the resistance gene(s) has not yet been completed. PI 567598B has previously been found to possess antibiosis resistance to soybean aphid, which is controlled by two recessive genes (Mensah et al., 2007). This research project was divided into three sections, each of which is presented as a chapter in this dissertation.

Objective 1: Discover the inheritance pattern of aphid resistance in PI 567585A, including the number and type of genes controlling resistance.

Objective 2: Identify and localize by traditional linkage mapping the aphid resistance gene(s) in PI 567585A in the soybean genome.

Objective 3: Map aphid resistance genes in PI 567598B using a new strategy: modified nested association mapping (MNAM), and compare the results obtained to those of traditional linkage mapping.

CHAPTER 1

LITERATURE REVIEW

THE SOYBEAN

The cultivated soybean, *Glycine max* (L.) Merrill. (2n=2x=40), is a species of legume native to eastern Asia. The genus *Glycine* consists of two subgenera, *Soja* and *Glycine*. The first subgenus *Soja* contains three species: *Glycine max* (L) Merrill., (the cultivated soybean) and *Glycine soja* (L) Sieb and Zucc., (the wild species). *Glycine soja* is the most likely potential progenitor of *Glycine max*. The second subgenus, *Glycine*, contains 12 wild, perennial species native to Australia and the Pacific area. The soybean is a highly self-pollinated species (Singh et al., 2007). Moreover, the cultivated soybean can be easily crossed within subgenus *Soja*, but not with the subgenus *Glycine* (Newell and Hymowitz, 1983). The soybean was first grown in the USA in 1765 (Hymowitz and Harlan, 1983). Many U.S. germplasm and ancestral genotypes camė from China, Korea and Japan (Li et al., 2001). During the past half century, the soybean, once an obscure forage crop, became a major grain crop in the United States (Singh et al., 2007).

The soybean is the second largest source of protein feed and vegetable oil in the world. The four major soybean-producing countries are the USA, Brazil, Argentina, and China, accounting for 90% of the global total production in 2005 (Workman, 2007). In 2007, soybean represented 56% of global world oilseed production, 32% of which was produced in the U.S. There are four major soybean production regions in the U.S.: North

Central (Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Ohio and Wisconsin), Northern Plain (Kansas, Nebraska and South Dakota), Southeast (Kentucky, North Carolina and Tennessee), and Delta (Arkansas, Louisiana and Mississippi) regions. Soybeans were planted on 30.6 million hectares in 2008, producing 80.54 million metric tons of soybeans in the U.S (Soy Stats, 2008).

THE SOYBEAN APHID

The soybean aphid, *Aphis glycines* Matsumura (Homoptera: Aphididae), is a native pest of soybean in eastern Asia. The soybean aphid (winged or wingless) is a small (<1/16" long when mature), yellow or yellowish green insect with two obvious black cornicles and pale cauda (Ragsdale et al., 2004). The soybean aphid was first reported in 2000 from the area of Wisconsin, northern Illinois, and Michigan. The pest spread over 21 states in the U.S. and three Canadian provinces (Hartman et al., 2001; Venette et al., 2004). Currently, the soybean aphid is one of the most significant pest insects in soybean production in North America.

The soybean aphid has a heteroecious holocylic life cycle, shuttling between its primary host, buckthorn (*Rhamnus cathartica*), and its secondary host, soybean. Soybean aphids overwinter as the egg stage on buckthorn. In the following spring, the eggs hatch and produce a few generations until the winged females (alatae) fly to soybeans. During the summer, soybean aphids commonly produce 15 generations with both wingless and

winged morphs throughout the whole growing season. In the soybean vegetative growth stages, soybean aphid colonies are found in partially expended young trifoliates, petioles, and stems. When the soybeans move to the reproductive stages, soybean aphids move over the whole plant, mature leaves, lateral branches, petioles, and pods. At the same time, the secreted honeydew on plants results in the production of sooty mold, which affects the photosynthesis and leads to the yield and seed quality loss. In the autumn when temperature and photoperiod are reduced, winged females (gynoparae) emerge on soybeans and disperse to find the primary host buckthorn. After gynoparae settle on the buckthorn, they develop into oviparae. Meanwhile, males also migrate to buckthorn to mate with oviparae. Finally they lay overwintering eggs on the buckthorn, starting the next life cycle in the following year (Ragsdale et al., 2004; Wu et al., 2004).

Severe soybean aphid infestations reduce soybean production directly by causing plant damage during feeding, such as wrinkled and distorted leaves, lower pod and seed counts, and reduced seed weight. In addition to the direct plant damage, soybean aphids have been reported to transmit diseases, including soybean mosaic virus (SMV), soybean stunt virus, soybean dwarf virus, abaca mosaic, beet mosaic, tobacco vein-banding mosaic virus, bean yellow mosaic virus, mungbean mosaic virus, peanut mottle virus, peanut stripe potty virus, and peanut mosaic virus (Wu et al., 2004).

The soybean aphid caused severe yield reduction in several north central states in 2003. An estimated 300,000 ha were affected in Michigan with a loss of $9 million

(DiFonzo, 2004). Soybean aphid damaged around 1.6 million ha of soybean in Minnesota with an estimated loss of $80 million (Associated Press, 2003). In Illinois, the estimated loss due to the infestation of 0.5 million ha soybean was $45 million (Steffey, 2004).

BIOTYPES OF SOYBEAN APHID

Biotype is a term employed to distinguish populations of insects or other organisms, which show different ability to attack plants due to diverse genetic variants. In many cases, resistance genes introduced by plant breeding often imposes selection pressure on insect populations, leading to the development of virulent insect biotypes (Gallum, 1972; Diehl and Bush, 1984). This means insects evolve to overcome the existing defense mechanism in plants. For example, the Hessian fly (*Mayetiola destructor*) that attacks wheat has twelve biotypes (Kudagamage et al., 1990; Ratcliffe et al.,, 2000); and the greenbug (*Schizaphis graminu)* has eight biotypes (Puterka et al.,, 1988).

The study in Michigan showed that the aphids overcame the resistance in 'Dowling' and 'Dowling' with the infestation of aphid colonies collected in 2006. PI 567598B and PI567541B retained the resistance to soybean aphid in greenhouse and field annual evaluations in Michigan (Mensah et al., 2007). In 2008, the research showed that there are at least two distinct biotypes of soybean aphid in North America: Ohio and Illinois isolates. The resistance genes *Rag1* from 'Dowling' and *Rag* from 'Jackson' were defeated by Ohio isolates, but were resistant to the Illinois isolate. PI 200538 and PI

567597C are resistant and PI 567541B is moderately resistant to both Ohio and Illinois aphid biotypes (Kim et al., 2008). These results indicated that aphids evolved spatial-temporally.

Integrated pest management (IPM) is a sustainable approach to manage crop pests by combining the use of chemical, natural biological and host plant resistance tactics, which minimizes economic, health and environmental risks.

CHEMICAL AND NATURAL BIOLOGICAL CONTROL STRATEGIES

There are several foliar insecticides registered for aphid control, which are all restricted use pesticides. Until now, the application of these insecticides is the only available and efficient way to control damage of soybean aphid in the commercial field. However, this approach is evaluated as cost-expensive, time-consuming and environmentally unfriendly. During the aphid outbreak in 2003, around 3 million ha of soybean were sprayed with pesticide to control the soybean aphid in the U.S (Landis et al., 2003). In Illinois, the cost of insecticide was $9 - 12 million for aphid control in the same year (Steffey, 2004).

Though insecticides work efficiently against soybean aphid, it is not recommended to apply them repeatedly as this can lead to insecticide resistance. In fact, many predators eat soybean aphids and keep aphid numbers in check naturally in some years preventing an outbreak. These predators include Asian lady beetle (*Harmonia axyridis*), lacewing

larvae (Neuroptera: Chrysopidae), syrphid fly larvae, minute pirate bugs (Hemiptera: Anthocoridae) and parasitoid Asian wasp (*Aphidius colemani*) (Fox et al., 2004). Among these predators, wasps have the most potential to be the natural biological control of soybean aphids. The Asian wasp can parasitize on soybean aphid and cause aphid mummies, is incapable of stinging people, and has a narrow biological host range. Other predators could not be applied in IPM because they either sting people or have a broader host range. As a strain of Asian wasp, *Binodoxys communis* was released for cage study in 2007 at 36 sites in seven Midwestern states: including Minnesota, Iowa, South Dakota, Wisconsin, Illinois, Indiana and Michigan. However, the parasitoid Asian wasp has not been officially released as natural predator in the field because of the difficulties to propagate and maintain the wasp number at a certain level under artificial conditions in each year (Ruth, 2007).

GENETIC CONTROL BY HOST PLANT INSECT RESISTANCE BREEDING

Host plant resistance breeding has enormous benefits as another component of IPM, in terms of investment return, reduced release of insecticide in the environment, and little concern about the population fluctuation of natural enemies each year (Li et al., 2007). Recently, host plant insect resistance has been developed in some new cultivars of rice, cotton, and vegetables, resulting in the reduced use of insecticides. Four components are involved in the host plant insect resistance breeding: 1) discovering host plant resistance

mechanisms; 2) determining the genetic inheritance of insect resistance; 3) identifying the insect resistance gene(s) in host plant; 4) integrating the resistance gene(s) into elite cultivars.

Discovering host plant insect resistance mechanisms

Insect resistance mechanisms are classified as tolerance, antibiosis and antixenosis (Painter, 1951). Both antibiosis and antixenosis describe the reaction of an insect to a plant, while tolerance resistance describes the reactions of a plant to insect attack. In tolerance mechanism, a plant can survive under the equal infestation pressure that would kill or severely injure other susceptible plants (Painter, 1951). In the antibiosis mechanism, the genetic properties of a plant reduce insect abundance by affecting the growth and production of the insect during feeding, leading to the decreasing plant damage. For example, first batch of Hessian fly larvae die after they start feeding on barley cultivars carrying the antibiosis resistance genes (Patterson et al.,, 1994). In the antixenosis mechanism, the insects feed and oviposit on a plant depending on the plant morphological characters, including color, leaf angle, odor, taste, and type of pubescence. For example the blue-green cultivars of peas are more favorable to the pea aphid than the yellow-green ones (Soroka and Mackay, 1991). Among these three resistance mechanisms, antibiosis was considered to be the only true form of host resistance because it involves antibiosis resistance genes in the host.

Both antibiosis and antixenosis deter insect feeding, so it is critical to separate these two resistance mechanism in insect resistance study. Choice and nonchoice tests have been extensively used to identify resistance, then the resistance type: either antibiosis or antixenosis (Mensah et al., 2005; Hill et al., 2004). First, a choice test is used to identify resistance, where aphids feed on their preferred hosts. But the test does not distinguish between the types of resistance. In nonchoice test, aphid movement is confined to a single host without preference. It helps distinguish antibiosis from antixenosis, nonhost preference. Antibiotic resistance source when identified can be used to develop host plant resistance.

In the U.S., the first four aphid resistance sources were reported in 2004. After screening 1,542 soybean accessions, 'Dowling', 'Jackson' and PI 200538 showed antibiotic resistance, and PI 71506 had antixenotic resistance (Hill et al., 2004). Both 'Dowling' and 'Jackson' are late maturity ancestral cultivars. In 2005, Mensah et al., evaluated 2,147 soybean germplams in choice tests and identified four new resistant accessions: PI 567541B, PI 567598B PI 567543C, and PI 567597C. The subsequent no-choice test showed that PI 567541B and PI 567598B possesses antibiotic resistance, while PI 567543C and PI 567597C have antixenotic resistance (Mensah et al., 2005). In 2006, Diaz-Montano et al. (2006) identified two antibiotic soybean entries: K1639 and Pioneer 95B97. In the following year, PI 239077 and PI 548664 were identified to have antibiotic resistance; while PI 595099, PI 436684, 'Perrin', and 'Tracy-M' have

antixenotic resistance (Hesler et al., 2007; Hesler and Dashiell, 2008). The latest aphid resistance sources were discovered by Mian et al. (2008a) after evaluating nearly 200 soybean genotypes by choice and no-choice tests in the greenhouse and field. PI 243540 showed antibiotic resistance, and PI 567301B and PI 567324 possessed antixenotic resistance. The currently available aphid resistance mechanism in different soybean accessions in the recent years is shown in Table 1.1. However, no commercial soybean cultivar with either partial or complete aphid resistance is currently available in the USA.

Determining the genetic inheritance of aphid resistance

Information on inheritance of resistance to insects, such as the number of genes and nature of gene action, can be utilized in selection of appropriate breeding methodology (pedigree, backcross or population improvement) to transfer resistance genes into elite cultivars. Classic genetic inheritance studies of insect resistance are based on the observation of phenotypes (resistance performance) in segregating populations. Commonly F_1 individuals, F_2 or backcross populations, and $F_{2:3}$ families are used to study the inheritance of insect resistance, such as whether the gene(s) is dominant or recessive, and how many genes are involved and whether resistance is qualitative or quantitative.

In several plants, the aphid resistance is mainly controlled by qualitative dominant/recessive genes, such as barley, cowpea, peach, wheat, and soybean. The aphid resistance in spring barley (*Hordeum vulgare* L.) is controlled by two dominant genes (Mornhinweg et al., 2002). The aphid (*Aphis craccivora* Koch) resistance in cowpea

(*Vigna unguiculata* L.) involves a single dominant gene. In the peach cultivar 'Rubira', the resistance to the green peach aphid (*Myzus persicae*) is controlled by a single dominant gene (Pascal et al., 2002). In wheat (*Triticum* spp.), eight independent dominant genes each confer resistance to the Russian wheat aphid (*Diuraphis noxia*) in different resistance source, while one recessive gene contributes to resistance in *Triticum tauschii* line SQ24 (Liu et al., 2006). The aphid resistance in 'Dowling', 'Jackson', PI 243540 and PI 200538 was found to be controlled by a single dominant gene (Hill et al., 2006a; Hill et al., 2006b; Kang et al., 2008; Hill et al., 2009). The latest genetic inheritance study of resistance in PI567598B and PI 567541B showed that two recessive genes involved in the aphid resistance (Mensah et al., 2008).

Identifying the aphid resistance gene(s) in soybean

The identification of aphid resistance genes has been important research challenge since 2000, due to the destructive soybean aphids. In 2007, two soybean aphid resistance genes *Rag1* and *Rag* were identified on linkage group (LG) M in soybean cultivars 'Dowling' and 'Jackson' respectively. *Rag1* was located 4.2cM from the Simple Sequence Repeat (SSR) marker Satt435 and 7.9cM from Satt463. *Rag* was mapped 2.1cM from Satt435 and 8.2cM from Satt463 (Li et al., 2007). In 2008, the aphid resistance gene *Rag2* in PI 243540 was positioned in the interval between the SSR markers Satt334 and Sct_033 on LG F (Mian et al., 2008b). In 2009, two resistance genes

in PI 567541B were closely linked to marker Satt299 or Satt435 on LG M, and to marker Satt649 or Satt343 on LG F (Zhang et al., 2009). The gene on LG F is far away from *Rag2*, while the position of the gene on LG M is in the similar region as the *Rag* or *Rag1*. Recently, the resistance gene of PI 200538 was mapped to the same region as *Rag2* (Hill et al., 2009), suggesting that PI 200538 may be an additional source of *Rag2*. The genetic allelic relationship among these genes on the same linkage group is still an enigma.

Transferring the aphid resistance gene(s) into elite lines

Two general categories of disease resistance have been recognized in plant: (1) qualitative resistance controlled by a single gene with large effect (resistance genes; R-genes) and (2) quantitative disease resistance (QDR) conditioned by multiple genes on quantitative trait loci (QTL), each with small individual effects and sensitive to environments (Poland et al., 2008).

Qualitative genes that provide high levels of resistance, are easily identified in genetic studies, and integrated into elite lines through pedigree or backcross breeding. For example, with the assistance of molecular markers linked to *Rag1*, the resistance gene was successfully backcrossed into the Midwest-adapted elite soybean lines without yield reduction (Kim and Diers, 2009). But they are subject to "break-down" due to the evolution of pest/pathogen populations. It means that the evolution of insect biotypes would overcome oligogenic resistance, resulting in a breakdown over a period of time.

Now, a total of three biotypes exist in North America: Michigan, Ohio and Illinois isolates. So it is necessary to consider the durability of an identified resistance resource. The period of aphid resistance is determined by the genetic inheritance of the resistance gene(s), such as the number of dominant/recessive gene(s) controlling the resistance. Generally, the resistance controlled by a single dominant gene is less durable than resistance controlled by multiple genes. For instance, the two genes-controlled resistance in PI 567598B and PI 567541B is more durable than single gene-controlled resistance in 'Dowling' and 'Jackson' (Mensah et al., 2007). In contrast, QDR tends to be more durable and favorable for breeding durable resistance cultivars, but no QDR was discovered to confer aphid resistance in soybean.

GENETIC MAPPING OF RESISTANCE GENE(S) IN CROP PLANTS

The goal of genetic mapping is to locate the genetic regions along the chromosomes which contain sequences that actively cause the phenotypic variation. This procedure statistically models the observed phenotypes in relation to genotypic information conveyed by molecular markers. Linkage analysis and association mapping are two widely used tools for the dissection of complex traits in genetic mapping (Ersoz et al., 2008; Zhu et al., 2008).

Population construction and phenotypic data

Genetic mapping in plants has been dominated by the linkage analysis of designed bi-parental populations with known pedigree structure. These bi-parental populations are derived from the cross of two distinct inbred lines, P_1 and P_2. They produce a heterozygous but homogenous F_1 –offspring. From the F_1, different types of population can be derived, including F_2, backcross (BC), double haploid (DH), or recombinant inbred line (RIL) populations (Sneller et al., 2009). Hundreds of linkage analysis studies have been done by establishing these kinds of bi-parental populations in various plant species over the past decades (Zhu et al., 2008; Sneller et al., 2009). In soybean aphid resistance studies, all resistance genes were identified by constructing bi-parental populations.

However, in principle, phenotypic data obtained from any type of population can be used for genetic mapping if the genetic variation exists within the population. Compared to linkage analysis within pedigrees, association mapping exploits the historical recombination in family based association population or classic association population (Crepieux et al., 2004; Parisseaux and Bernardo, 2004; Zhang et al., 2004; Bernardo and Yu, 2007), resulting in higher power of detection in specific regions of DNA (Zhu et al., 2007). A family based association population is composed of unrelated families, which are powerful for gene mapping of complex diseases mainly in human. Classic association population is equal to a natural population, which is a random sample of individuals from species population. The classic association population is used widely

in association mapping of crops to understand the diversity of germplasm, such as maize, barley, sorghum and wheat (Camus-Kalandalvelu et al., 2006; Murray et al., 2009; Breseghello and Sorrells, 2006). For example, association mapping provides an efficient approach to relate genotypes to complex quantitative traits in hexaploid wheat. In 2006, association mapping of kernel size and milling quality was performed on a selected sample of 95 elite cultivars from soft winter wheat germplasm by using SSR markers. The association mapping results showed not only more QTLs, but also agreement with previous linkage analysis at certain significant SSR marker loci on three chromosomes (Breseghello and Sorrells, 2006). Later, a population of 44 modern European winter wheat varieties was studied by association mapping for the association between *Stagonospora nodorum* blotch resistance and markers mapped in the region of QSng.sfr-3BS. The results showed that the association mapping population had at least a 390-fold higher resolution compared to the traditional RIL populations (Tommasini et al., 2007). The association mapping was also used to assess the genetic diversity of biotype2 Russian wheat aphid (RWA2) resistance within 71 bread wheat *(T. monococcum)* accessions. New QTLs were identified by association mapping, compared to previous linkage analysis (Peng et al., 2009). Thus, as the supplementary of linkage analysis, association mapping emerged to exploit trait variation with sufficient recombinant events within a more flexible population construction, such as a natural population, or a diverse collection of germplasm (Zhu et al., 2008).

Genotypic data

There are numerous different types of molecular makers that have been used to obtain genotypic data in genetic mapping, including Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR) and Single nucleotide polymorphism. Each type of molecular marker has its own advantages and disadvantages. For example, SSR markers are most popular in current soybean genetic studies due to desirable characteristics of co-dominance, highly polymorphic and sufficient genome coverage. The latest version of the SSR-based soybean linkage map was released in 2004 (Song et al., 2004). This integrated genetic map covers 2,523.5 cM of soybean genome across 20 LGs that contained 1,015 SSR markers. More recently, the appearance of high-throughput and inexpensive SNP genotyping platforms stimulated the development of SNP markers in soybean genome. SNPs were first discovered by the resequencing of sequence tagged sites (STS) by Choi et al., (2007). As a newly informative genetic marker, SNP includes single base changes, insertions/deletions (indels). A total of 1,141 sequence-based SNP markers were used to fill the gaps (>5 cM) in the pre-existing SSR-based map.

The new SSR/SNP based genetic map of soybean genome provides a crucial resource for quantitative trait locus discovery, map-based cloning, and marker assisted selection in cultivar improvement. Moreover, the U.S. Department of Energy Joint

Genome Institute (DOE JGI) completed the preliminary assembly and annotation of the soybean genome, *Glycine max*. This will accelerate SNP discovery and the construction of a dense SNP-based soybean genome map (Hyten et al., 2007a, 2007b).

Linkage analysis

Linkage analysis looks for non-random co-segregation of marker alleles and trait within pedigrees (F_2, BC, DH, RIL populations). For linkage analysis, genotype and phenotype are integrated into the different statistical models for QTL detection, such as single-marker regression, simple interval mapping (SIM) (Lander and Botstein, 1986), composite interval mapping (CIM) (Zeng, 1994) and multiple interval mapping (MIM) (Kao et al., 1999). The linkage analysis has been widely applied in identification of aphid resistance genes in soybean since 2000. The linkage mapping populations are F_2, $F_{3:4}$, $F_{4:5}$ RILs populations which were derived from resistant and susceptible parents, where SIM, CIM and MIM were applied to detect the QTL positions.

However, limited numbers of meioses exist within families and pedigrees in few generations for linkage analysis, which is suitable to detect QTL in genome-wide with low resolution at the order of megabases (Thornsberry et al., 2001). Until now, a few number of QTLs identified in linkage analysis were tagged at the gene-level.

Association mapping

1) Linkage disequilibrium

Association mapping was established on the basis of linkage disequilibrium (LD) concept, which is the non-random co-segregation of alleles at two loci. The precision of association mapping is determined by the rate of decay of LD with physical distance. The rate is highly variable among species, and even among different genomic regions of the same species, such as coding and non-coding regions. The relationship between LD and physical distance determines the marker density required for genome scan, and the maximum resolution for phenotype genotype association in the study population (Veyrieras et al., 2007). As an autogamous crop, the LD structure of soybean has been analyzed in three genomic regions, and LD ranged from 336 to 574 kb. The highly variable levels of LD were discovered in the wild ancestors, landraces and the elite cultivars. In *G. soja*, LD extends over 100 kb. But LD coverage is highly variable in the landrace and elite cultivars, expanding from 90 to 574 kb because of the domestication and increased self-fertilization (Hyten et al., 2007a).

2) Population structure in association mapping

A population is said to be structured if individuals or families deviate from Hardy-Weinburg equilibrium due to domestication, natural or artificial selection, and admixture of populations. Association mapping investigate the association between genetic diversity and phenotypic variation. But the association between population structure and trait variation can complicate the identification of accurate correlations in association mapping because of the indirect association between neutral polymorphism

and phenotypic variation. Moreover, the trait of interest determines the magnitude of variation could be explained by population structure in wheat, 4-6% for kernel composition, or up to 51% for flowing time (Veyrieras et al., 2007).

3) Statistics in association mapping

Association mapping investigates the association between genetic diversity and phenotypic variation. This approach has higher possibilities of type I and type II errors, which means increased false positives and reduced power in association mapping compared to biparental linkage analysis (Breseghello and Sorrells, 2006). So many statistical challenges cumber the dissection of phenotypic variation of complex traits of interest.

The false positive rate (Type I error) genome-widely is caused by the multiple hypotheses testing during the whole genome association mapping, whereby some random or indirect associations occur in large collection of molecular markers. Type I error rate for multiple testing can be controlled genome widely with the Bonferroni correction or false discovery rate (FDR) (Benjamini and Yekutieli, 2005). Generally, the Bonferroni correction is more conservative and reduces the power of detecting real association between the polymorphism and the traits. The FDR controls the expected proportion of false positives in the whole set of positive results from the multiple testing. So it is a more flexible procedure with greater statistical power. In the FDR approach, the q-value of a test measures the proportion of false positives incurred when a particular test is

called significant. The Q-value package estimates the q-values from the list of p-value of the multiple testing, estimates a cutoff for a particular FDR, and estimates an FDR for a particular cutoff (Chen and Storey, 2006).

Type II error, or false negative is attribute to population structure and familiar relatedness among or within different subpopulations. Thus, association mapping has limited application in detecting rare variant or genes existed within subpopulations, but inconsistently fixed among populations (Zhang et al., 2004; Veyrieras et al., 2007). So it is necessary to discover the hidden effect of population structure before the investigation of candidate polymorphism contribution to phenotypic variation in a collection of diverse materials. Recently, some software or methodologies were developed to understand population structure by analyzing molecular data, such as STRUCTURE (Hubisz et al., 2009), principal component analysis (PCA), and Ward.

Nested association mapping in plants

Nested association mapping was developed for dissecting the genetic inheritance of complex quantitative traits in maize, which combines the advantages of linkage analysis and association mapping in a single population (Yu et al., 2008). This NAM population was developed by crossing the common parent B73 with 25 different founder parents. Each individual obtained from 25 F_2 populations were self-pollinated four generations, producing a total of 5000 recombinant inbred lines (RIL). This RIL population can be used for cursory QTL detection by linkage mapping with low-resolution markers,

followed by high-resolution association mapping with high-density maker, such as SNPs. As a permanent and stable genetic mapping resource, the NAM population can be evaluated for many quantitative traits in multiple environments, producing accurate estimates of significant allelic effects, epistasis, pleiotropy and genotype-environment interaction. This NAM methodology has been applied in the dissection of quantitative traits in crop plants, such as northern leaf blight resistance and flowering time in maize (Poland et al., 2009; Buckler et al., 2009).

MOLECULAR BASES FOR RESISTANCE (R) GENES

Five different classes of R-genes have been identified in plants based on the combination of structural motifs. Class I contains a serine-threonine kinase (STK) catalytic domain, such as *Pto* conferring resistance to the bacterial pathogen *Pseudomonas syringae* in tomato (Tang et al., 1999). As the largest two classes, the second and third class both are composed of a putative nucleotide binding site (NBS), leucine-rich repeats (LRRs), but different in the presence of either a coiled-coil (CC) domain or a Toll/Interleukin-1 cytoplasmic receptor (TIR) at the amino terminus. Being intracellular proteins, no transmembrane (TM) domain exists in Class I through 3 R-gene families. *Mi-1* belongs to class 2 R-gene family, conferring resistance to root-knot nematodes and potato aphids in tomato (Vos et al., 1999). Class 4 R-genes have a TM and an extracellular LRR without NBS. The coded protein in the fifth R-gene class

possesses an extracellular LRR, a TM and a cytoplasmic STK domain. For example the rice R-gene *Xa21* confers *resistance* against the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (Khush et al., 1990).

Hundreds of NBS-LRR resistance gene analog (RGA)s were isolated and sequenced, and genetically mapped to 8 of the 26 linkage groups of the soybean genetic map, including groups C_2, D_1, H, J, L, M, N and P (Kanazin et al., 1996; Yu et al., 1996; Graham et al., 2000). The R-genes often possess similar sequence and are physically clustered within a close distance on the same linkage group. Five classes of RGAs were co-localized as a large cluster on LG J (Kanazin et al., 1996). LG F is another important region for disease resistance genes in soybean, where several RGAs related to virus, bacteria fungus and nematode resistance are mapped (Jeong et al., 2001). All this information matched well with the available locations of aphid resistance genes in soybean.

The NBS-LRR gene family is organized and evolves through a process of birth-and-death, supercluster formation, and adaptive selection. That means the superclusters are initiated from deleterious mutation, repeated gene duplication, intra/inter-cluster recombination, transposition, or genome rearrangement. Then the cluster/superculster are maintained intact or lost from the cluster/supercluster in natural or artificial selection. For example, the conditional resistance to the soybean mosaic virus (SMV; Potyvirus) resistance involves multiple-allelic gene on *Rsv*, which is tightly linked

to a NBS/LRR gene cluster on the LG F. The study showed that recombination within the cluster can condition the specific resistance for all SMV strains in soybean (Hayes et al., 2004).

MOLECULAR BREEDING PRACTICE

Marker-assisted selection (MAS) is a process widely used to accelerate plant breeding through early generation selection, whereby molecular markers are used for indirect selection of traits of interest. The first step of MAS is to map the gene or QTL by genetic mapping, and then the closely linked flanking markers in the candidate gene region are used for MAS. MAS is based on linkage analysis, which has been developed for most crop plants to locate gene/QTL regions where molecular markers co-segregate with traits of interest. Linkage analysis based on MAS is most likely to be used for within-family selection in a limited number of elite families. The limitations of this approach include the low resolution of MAS, inconsistent QTL detection in different genetic background and environments. These properties prevent the development of universal markers for marker-assisted selection at multiple-population or germplasm level. So gene-assisted selection (GAS) emerged to exploit the direct association between gene(s) and trait based on association mapping. High resolution of marker-trait associations is detected in association mapping due to the advances in high-throughput

sequencing and SNP genotyping platform. The closely linked markers (or genes) are transferrable across multiple families, natural population and even species.

Progress of using MAS already existed in the area of quantitative disease resistance. For an instance, the dominant gene *Xa21* was located on chromosome 11, conferring broad spectrum resistance to most isolates of *Xanthomonas oryzae* pv. *oryzae* (Ikeda et al., 1990, Khush et al., 1990). As a crucial gene for bacteria resistance improvement in rice, *Xa21* has been used in marker-assisted breeding by tagging with molecular markers (Ronald et al., 1992; Sharma et al., 2001, Singh et al., 2001, Sridhar et al., 2001). In current soybean aphid resistance studies, the close linkage SSR markers were used to breed new productive resistant soybean cultivar (Kim and Diers, 2009).

Table 1.1 List of soybean aphid resistance sources available as of 2009.

Resistance Mechanism		Reference
Antibiotic resistance	Antixenotic resistance	
'Dowling'	PI 71506	Hill et al., 2004
'Jackson'		
PI 200538		
PI 567598B	PI 567543C	Mensah et al., 2005
PI 567541B	PI 567597C	
K1639		Diaz-Montano et al., 2006
Pioneer 95B97		
PI 230977	PI 595099	Hesler et al., 2007
PI 548664	PI 436684	Hesler and Dashiell, 2007
	'Perrin'	
	'Tracy-M'	
PI 243540	PI 567301B	Mian et al., 2008a
	PI 567324	

Table 1.2 List of aphid resistance genes identified in soybean accessions available as of 2009.

Source	Gene	Linkage group	Flanking Markers	% variation explained	Reference
'Jackson'	*Rag*	M	Satt435~Satt463	-	Li et al., 2007
'Dowling'	*Rag1*	M	Satt435~Satt463	-	Li et al., 2007
PI 243540	*Rag2*	F	Satt334~Sct_033		Mian et al., 2008b
PI 567541B	-	F	Satt299~Satt435	50.3%	Zhang et al., 2009
	-	M	Satt649~Satt343	29.5%	Zhang et al., 2009
PI 200538	-	F	Satt334~Sct_033	-	Hill et al., 2009

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CHAPTER 2

INHERITANCE OF RESISTANCE TO THE SOYBEAN APHID IN SOYBEAN PI 567585A

Abstract: The soybean aphid (*Aphis glycines* Matsumura) is an important insect pest of soybean [*Glycine max* (L.) Merr.]. Soybean plant introduction (PI) 567585A has shown strong resistance to soybean aphids. The objective of the study was to determine the inheritance of aphid resistance in PI 567585A. This resistant soybean PI 567585A was crossed with two susceptible soybean cultivars, 'Skylla' and IA2070. The parents, F_1, F_2 plants and $F_{2:3}$ families were evaluated for aphid resistance in the field during the summer of 2008 and 2009. All F_1 plants exhibited phenotype intermediate between the resistant and susceptible parents. The observed segregation ratios in the two F_2 populations (070082-1 and 070016-1) fitted a segregation ratio 1:2:1 (Resistant: Intermediate: Susceptible). The $F_{2:3}$ families also fitted the segregation ratio of 1:2:1 (Resistant: Segregating: Susceptible). These data indicated that the aphid resistance in PI 567585A was controlled by one major co-dominant gene.

Abbreviations: LG: linkage group; PI: plant introduction;

Key word: soybean aphid resistance, co-dominant, PI 567585A, segregation ratio

The soybean aphid (*Aphis glycines* Matsumura) was first found in the United State in 2000 (Hartman et al., 2000). Since then, it has been reported in 21 states in the United States and three provinces in Cananda (Venette and Ragsdale, 2000; Ragsdale et al., 2004). In Michigan, an estimation of 740,000 hectares was affected and cost $9 million (DiFonzo, 2004). This insect sucks sap from plants, excrete honeydew on plants. The sooty mold also develops on the honeydew which the aphids secrete, inhibiting the photosynthesis process. Soybean aphids were reported transmitting viruses found in soybean, potato, dry bean and vine crops (Mian et al., 2008a). It caused severe yield reduction in soybean in several north central states in 2003 (Zhang et al., 2009).

Soybean aphids are generally controlled by application of foliar insecticides, though it is costly and not environmentally friendly. A better alternative will be the use of aphid resistance cultivars. However, no commercial aphid resistant soybean cultivar is currently available in North America. Several aphid resistance sources were discovered in early and late maturity germplasm, such as 'Dowling', 'Jackson', PI 567598B, PI 567541B, PI 243550, and PI 200538 (Hill et al., 2004; Mensah et al., 2005; Mian et al., 2008a; Hill et al., 2009). Recently we identified a new source of aphid resistance, PI 567585A (Dechun Wang, unpublished data). PI 567585A is a maturity group II germplasm accession originated from Shandong, China (Hill et al., 2005). It showed resistance against the soybean aphid in both choice and non-choice tests.

For some of existing aphid resistance sources, the inheritance patterns and the location of resistance genes have been investigated since 2006. The aphid resistance in 'Dowling' and 'Jackson' is controlled by a single dominant gene. This gene is located on linkage group (LG) M and named as *Rag1* and *Rag*, respectively (Hill et al., 2006a; Hill et al., 2006b; Li et al., 2007). Resistance in both PI 567598B and PI 567541B accessions is conditioned by two recessive genes (Mensah et al., 2008). The resistance genes of PI 567541B were recently mapped on LGs M and F (Zhang et al., 2009). The antibiosis resistance in PI 243550 and PI 200538 accessions was also controlled by a single dominance gene designated *Rag2*, which is located on LG F (Kang et al., 2008; Mian et al., 2008b; Hill et al., 2009). The inheritance of aphid resistance in PI 567585A is unknown. The objective of this study is to determine the inheritance of aphid resistance in PI 567585A.

Materials and Methods

Population construction and aphid resistance evaluation

PI 567585A was crossed with 'Skylla' and IA2070. PI 567585A is resistant to soybean aphids, and both 'Skylla' and IA2070 are susceptible cultivars. F_1 plants and F_2 populations were developed from the crosses Skylla x PI 567585A and IA2070 x PI 567585A (070082-1 and 070016-1). The parental lines, F_1 plants, and F_2 populations were evaluated during the summer of 2008 in the field on the Agronomy Farm of

Michigan State University (MSU). The field evaluation of soybean aphid resistance was carried out in a 12.2- x 18.3-m aphid- and predator- proof cage. Fourteen days after planting, each plant was inoculated with two wingless aphids (Mensah et al., 2005) at the V2 stage (Fehr and Caviness, 1977). All aphids used in inoculation were collected from nearby naturally infested soybean fields. Three parents, PI 567585A, Skylla, and IA 2070 were planted 6.0 cm apart at row width of 38 cm with three replications. F_1 and F_2 plants were planted 5.0 cm apart at row width of 38 cm with no replications. Each parental, F_1 and F_2 plant was rated for aphid resistance 21 days after inoculation using the modified half step scale ranging from 0 to 4 as described by Mensah et al. (2008).

Seeds from individual F_2 plants in populations 070082-1 and 070016-1 were harvested individually during the fall of 2008. Depending on seed availability, F_2 plants with a minimum of 11 progenies, resistant, susceptible or intermediate to soybean aphids, were chosen for further aphid resistance study during the summer of 2009. One hundred and fifty eight $F_{2:3}$ families were obtained from the 070082-1 F_2 population, and 58 $F_{2:3}$ families were collected from the 070016-1 F_2 population. $F_{2:3}$ plants were planted 3.0 cm apart with no replication in the field cage during the summer of 2009. The soybean aphid resistance was scored for each $F_{2:3}$ family on a row basis as described before.

Statistical Analysis

Chi-square tests were performed to test the goodness of fit of observed segregation ratios for F_2 populations and $F_{2:3}$ families with the expected genetic ratios. In order to analyze the segregation in F_2 populations, each individual plant was classified as resistant if it had a rating equal to or lower than the resistant parent, or as susceptible if it had a rating equal to or higher than the susceptible parents, or as intermediate if the observed phenotype of an individual plant was between the resistant and susceptible parents. Segregation among $F_{2:3}$ progeny was analyzed by classifying each family into three groups: homozygous resistant (all observed ratings of one family were equal to or lower than resistant parent), segregating (all observed ratings of one family were segregating for resistant, heterozygous and susceptible phenotypes), and homozygous susceptible (all observed ratings of one family were equal to or higher than susceptible parents).

Results and discussion

Aphid resistance evaluation for F_1 plants

The aphid resistance ratings for PI 567585A plants were 0.5 or 1.0., and 3 or 3.5 for Skylla and IA 2070 plants. All F_1 plants from the two crosses demonstrated a phenotype intermediate between the resistant and susceptible parents ranging from 1.5 to 2.5 (Table 2.1). Thus, an individual plant in F_2 populations with a rating of 1.0 or less was classified as resistant, a plant with a rating larger than 3.0 was regarded as susceptible, and a plant with a rating ranging from 1.5 to 2.5 was considered intermediate.

Segregation analysis for aphid resistance in F_2 populations

The frequency distributions of the aphid resistance ratings in the two F_2 segregating populations are shown in Figure 1. For the F_2 population of 070082-1, the segregation was 61 resistant plants, 115 intermediate plants and 45 susceptible plants. The segregation ratio fits a 1:2:1 ratio (P=0.2614) (Table 2.2). For 070016-1 F_2 population, the segregation of resistant, heterozygous, and susceptible plants was 21:51:14, fitting a 1:2:1 ratio (P=0.1277) (Table 2.3). Thus, resistance to the soybean aphid showed a qualitative character in two F_2 populations. Moreover, both segregation ratios of F_2 populations fit the expected 1:2:1 ratio, indicating a major co-dominant gene controlling the aphid resistance in PI 567585A.

Segregation analysis for aphid resistance in $F_{2:3}$ families

A total of 158 'Skylla' x PI 567585A $F_{2:3}$ families were collected for the progeny tests based on the requirement that they had produced a minimum of 11 seeds to allow adequate statistical analaysi. The ratio of 41:84:33 fits a 1:2:1 resistant/intermediate/susceptible ratio (P=0.4860), fully representing the 070082-1 F_2 population. The $F_{2:3}$ progeny test of 070082-1 F_2 plants showed a ratio of 38:75:45 homozygous resistant/segregating/homozygous susceptible, significantly fitting the ratio1:2:1 (P=0.5989) expected for a monogenic co-dominant gene (Table 2.4). For the 070016-1 F_2 population, 58 $F_{2:3}$ families were collected for the progeny test. The ratio of

resistant/intermediate/susceptible, which was found to be 17:26:15, fits the expected 1:2:1 ratio (P=0.6843), fully representing the 070016-1 F_2 population. The results showed a ratio of 16:28:14 homozygous resistant/segregating/homozygous susceptible, which significantly fits the ratio1:2:1 (P=0.9017) expected for a monogenic co-dominant gene (Table 2.3).

Dominant, recessive and co-dominant nature of aphid resistance

Both dominant and recessive genes have been identified for the aphid resistance in plants. In wheat germplasm accessions, one recessive gene and eight dominant genes have been identified for Russian wheat aphid resistance, and are generally qualitatively inherited (Liu et al., 2001). In alfalfa and sweet clover, a single dominant gene controls resistance to the pea aphid, *A. pisum* (Glover and Stanford, 1966), and the sweet clover aphid, *Therioaphis riehmi* (Manglitz and Gorz, 1968). Spotted alfalfa aphid resistance in alfalfa is controlled by several genes, suggesting the quantitative inheritance of resistance (Glover and Melton, 1966). In some *Solanum* species, resistance to the green peach aphid, *Myzus persicae* exhibits a partially dominant inheritance (Sams et al., 1976). Moreover, in the soybean cultivar 'Dowling', 'Jackson', PI 200538 and PI 243550, resistance to soybean aphid is controlled by a single dominant gene (Kang et al., 2008, Hill et al., 2009). However, some insect resistance is conferred by co-dominant or recessive gene(s). For example, the resistance genes underlying *rhg1* in soybean were found to be dominant, recessive and co-dominant in the study of the Hg type resistance to the soybean cyst

nematode (*Heterodera glycines*) (Meksem et al., 2001; Afzal et al., 2009). A monogenic co-dominant gene controlled resistance to the cyst nematode (*Heterodera sacchari*) in African rice, *Oryza glaberrima* (Lorieux et al., 2003). And two recessive genes control the aphid resistance in soybean accessions PI 567598B and PI 567541B (Mensah et al., 2008).

In this study, aphid resistance in PI 567585A was shown to be controlled by a single co-dominant gene controlling the aphid resistance. The inheritance pattern is different to other host-plant aphid resistance sources. Louriex (2003) mentioned that different phenotyping methods were applied to identify the possible co-dominant inheritance as dominant in numerous pathogen resistance studies. In previous inheritance studies of 'Dowling', 'Jackson', PI 200538 and PI 243540, researchers used the 1-4 nonparametric ordinal rating or 1-5 modified scoring (Hill et al., 2006a; Hill et al., 2006b; Mian et al., 2008; Hill et al., 2009) because the segregating populations only showed two distinct parental resistance phenotypes without any intermediate characteristics. In our study, the F_2 segregation populations showed intermediate phenotypes between two distinctive parents, exhibiting discontinuous normal distributions. The 0-4 half-step rating scale can effectively separate the variable aphid damage on individual plants in the field, allowing for the clarification of plants into different scales. Hill et al. (2006) also pointed that there would be more variability in aphid colonization on plants in the field compared with tests carried out in the greenhouse. This inheritance study was completed in the field,

compared to the previous utilized inheritance studies in greenhouses. Thus, the 0-4 half-step rating scale was necessarily recruited in the field to evaluate variable aphid damaging on individual plants.

In summary, a single co-dominant gene was discovered for soybean aphid resistance in soybean accession PI 567585A. As a monogenic resistance source, it can be easily introgressed into elite lines through backcross or pedigree breeding. However, it likely will be overcome during a period of time due to evolution of insect biotype. Pyramiding resistant genes is an effective strategy against the target insect/pathogen population, which has been studied in soybean and rice (Hittalmani et al., 2000; Maroofa et al., 2008). Our next step is to map the resistance gene in PI 567585A, and discover whether it co-localize with the current resistance gene(s) located on LG F, J or M, or it is a new gene located elsewhere on the soybean genome. Ultimately, the genetic mapping of the resistance gene in PI 567585A will hasten the selection of aphid resistance in breeding program through use of marker assisted selection methods.

Table 2.1 Observed soybean aphid resistance rating for parental and F_1 plants 21 days after aphid inoculation in field in 2008 summer.

Genotype	Total number of plants tested	Number of plants	Mean rating
PI 567585A	8	8	0.8
Skylla	10	0	3.5
IA2070	11	0	3.4
(Skylla x PI 567585A) F_1	5	5	2.4
(IA2070 x PI 567585A) F_1	5	--*	--*

*: The data were unavailable for the F_1 plants from IA 2070 x PI 567585A due to weakness and death in field.

Table 2.2 Segregation of soybean aphid resistance in F_2 populations derived from different crosses.

Population ID	Susceptible Parent	Resistant Parent	Total no. of plants	Observed			$X^2_{1:2:1}$	$P_{1:2:1}$
				R	I	S		
070082-1	Skylla	PI 567585A	222	61	116	45	2.683	0.2614
070016-1	IA2070	PI 567585A	86	21	51	14	4.116	0.1277

R: resistant with score 0.5 or 1.0

I: intermediate between resistant and susceptible with score ranging 1.5-2.5

S: susceptible with score 3.0, 3.5 or 4

Table 2.3 Segregation of $F_{2:3}$ progenies from PI 567585A x Skylla and IA2070 F_2 plants for aphid resistance rating.

Population No.	F_2 plant phenotype	F_2 plant genotype	No. of $F_{2:3}$ families	$X^2_{1:2:1}$	$P_{1:2:1}$
070082-1	Resistant	RR	17		
		Rr	20		
		rr	4		
	Intermediate	RR	19		
		Rr	44		
		rr	21		
	Susceptible	RR	2		
		Rr	11		
		rr	20		
	Total			1.025	0.5989
070016-1	Resistant	RR	7		
		Rr	10		
		rr	0		
	Intermediate	RR	9		
		Rr	15		
		rr	2		
	Susceptible	RR	0		
		Rr	3		
		rr	12		
	Total			0.207	0.9017

RR: homozygous resistant (all $F_{2:3}$ plants in an individual family are resistant)

Rr: segregating progenies (all $F_{2:3}$ plants in an individual family are a segregating resistant, heterozygous and susceptible)

rr: homozygous susceptible (all $F_{2:3}$ plants in an individual family are susceptible)

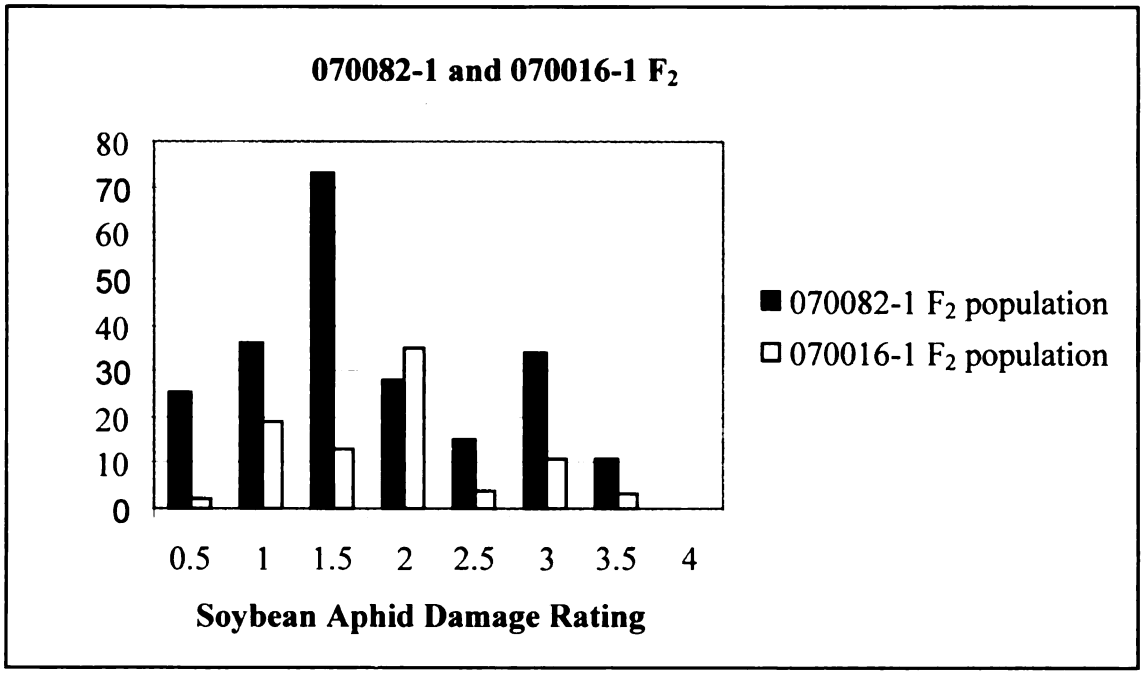

Figure 2.1 Frequency distribution of soybean aphid resistance rating scores for F_2 populations 070082-1 and 070016-1, respectively.

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CHAPTER 3

GENETIC LINKAGE MAPPING OF THE SOYBEAN APHID RESISTANCE GENE IN PI567585A

ABSTRACT

The soybean aphid (*Aphis glycines* Matsumura) is an important insect pest of soybean [*Glycine max* (L.) Merr.] in North America. In our previous study, PI 567585A was shown to possess soybean aphid resistance controlled by a single co-dominant gene. The objective of this study was to determine the genetic basis of aphid resistance in PI 567585A. A mapping population of 158 $F_{4:5}$ recombinant inbred lines (RILs) derived from the cross between PI 567585A (resistant to soybean aphid) and 'Skylla' (cultivar susceptible to soybean aphid) was evaluated for aphid resistance in both the greenhouse and field in 2009. Broad-sense heritability estimate of aphid resistance in the field trial was 95.5%. The single aphid resistance gene was mapped in an interval between Satt674 and Sct_065, simple sequence repeat (SSR) markers on chromosome 16 (linkage group J) using the composite interval mapping method. The locus explained 93.1% of the phenotypic variation in the field trial, and is located in the same genomic region as *Rag3*. This single aphid resistance gene in PI 567585A was confirmed in another $F_{3:4}$ RIL population derived from a cross between PI 567585A and a susceptible parent IA2070. The SSR markers linked to aphid resistance in PI 567585A discovered in this study,

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along with other independent aphid resistance genes from diverse germplams, could be used to pyramid multiple genes into a soybean cultivar for more durable aphid resistance.

INTRODUCTION

Soybean aphids (*Aphis glycines* Matsumura) are native to eastern and southeastern Asia. The insects were first reported in North America in July 2000 (Hartman et al., 2001). Since it was detected, this new pest has rapidly increased to very high population densities and spread to 21 states in the U.S. and three provinces in Canada (Zhang et al., 2009a). High populations of soybean aphids suck sap from soybean plants, secret honeydew on plants during the early reproductive stages, causing reduced pod set. In addition, soybean aphids can transmit soybean viruses (DiFonzo and Hines, 2002).

Soybean growers typically control soybean aphid by applying foliar insecticides, which increase the production costs and are released in the environment. The best alternative control strategy would be the utilization of aphid resistant cultivars; yet, there are no commercial aphid resistant soybean cultivars available in North America. To date, several aphid resistance sources have been found in soybean accession. In the genetic inheritance pattern of resistance has been determined in seven genotypes, including 'Dowling', 'Jackson', PI 243550, PI 200538, PI 567598B, PI 567541B, and PI 567543C. In six genotypes (with the exception on of PI 567598B), the location of the resistance gene(s) has been mapped. Single dominant genes *Rag1* and *Rag* on

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chromosome 7 (LG M) control aphid resistance in 'Dowling' and 'Jackson', respectively (Hill et al., 2004; Hill et al., 2006a; Hill et al., 2006b; Li et al., 2007). Aphid resistance in PI 243550 and PI 200538 is also controlled by a single dominant gene designated *Rag2*, which is located on chromosome 13 (LG F) (Kang et al., 2008; Mian et al., 2008a; Mian et al., 2008b; Hill et al., 2009). Two recessive genes are involved in the resistance of both PI 567598B and PI 567541B (Mensah et al., 2008). The two resistance genes of PI 567541B have been recently mapped on LGs M and F, namely *rag1_C* and *rag4* (Zhang et al., 2009a). *rag1_C* was mapped to the same genome region as *Rag1*, while *rag4* was distantly located from *Rag2*. Most recently, a major gene, *Rag3* on chromosome 16 (LG J), was identified in the aphid resistance in PI 567543C (Zhang et al., 2009b).

Clustering and sequence similarity of different resistance gene analog (RGA) classes are known from other disease and insect resistance studies in plants. Genetic mapping of nine classes of RGA located them on eight linkage groups of the soybean genetic map, dispersing singly or in clusters along several LG, such as C_2, D_1, H, J, L, M, N and P. Five classes of RGAs were co-localized as a large cluster on LG J (Kanazin et al., 1996). LG F is another known important region where disease resistance genes are clustered in soybean, where several RGAs related to virus, bacteria, fungus, and nematode resistance are mapped (Jeong et al., 2001). All of this information matches well with the available locations of aphid resistance genes in soybean. Therefore, LG F, J and M in soybean genome were given priority for mapping new aphid resistance locus.

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A new source of aphid resistance was identified in PI 567585A, a maturity group II germplasm accession originated from Shandong, China (Hill et al., 2005). It showed resistance against the soybean aphid in both choice and non-choice tests (Dr. Dechun Wang, unpublished data). In a previous study, one co-dominant gene was determined to control the inheritance of aphid resistance in PI 567585A (Liu et al., unpublished data). However, the location of the aphid resistance gene in PI 567585A is unknown. Thus, the objective of this study was to map and validate the aphid resistance gene in PI 567585A with linked SSR markers.

Materials and Methods

Plant materials and aphid resistance evaluation

A mapping population of 158 $F_{4:5}$ lines (070082) was developed from the cross of PI 567585A x 'Skylla' by single seed descent. PI 567585A possesses antibiosis resistance to the soybean aphid (Dechun Wang, unpublished data). The Chinese cultivar name of PI 567585A is 'Ri Zhao Huang'. The morphological and agronomic traits of PI 567585A are listed by Hill et al. (2005). 'Skylla' is an aphid-susceptible soybean variety (Wang et al., 2006).

Based on the heritability of aphid resistance shown in previous experiments (Zhang et al., 2009a), a single trial was carried out in the greenhouse and two replications were conducted in field. The greenhouse trial was initiated in the Plant Science Greenhouse at

Michigan State University (MSU) in East Lansing, Michigan. Eight seeds per line or parent were planted in a plastic pot, which is 210 mm in diameter and 125mm deep. In a completely randomized design (CRD), two parents and the mapping population were set on the bench without replication. The temperature was maintained at 26/15°C day/night with 14-h supplemental lighting provided by sodium vapor lamps. In the summer of 2009, the field evaluation of soybean aphid resistance was carried out in a 12.2 x 18.3m aphid- and predator- proof cage (Redwood Empire Awning Co., Santa Risa, CA) on the Agronomy Farm of MSU. The parental plants were planted randomly in the field, 5.1 cm apart, with two replications. Depending on the seed availability, 4 to 16 seeds per line were planted in a single row plot, 60cm long with a row spacing of 60cm. The average number of plants per recombinant inbred line was around nine with most plots having at least eight plants. Similarly, CRD was used to arrange the whole $F_{4:5}$ population and its parents in the field plots with two replications.

In both greenhouse and field trials, each plant was inoculated at the V2 stage with two wingless aphids. A single aphid clone was collected from a naturally infested field at the MSU Agronomy Farm in summer 2008, and maintained in an isolation chamber in the greenhouse for the inoculation of plants in the greenhouse trial in 2009 spring. The soybean aphids used for inoculation in the field trial were collected from a naturally infested field on the MSU Agronomy Farm in 2009 summer. The $F_{4:5}$ mapping population and parental plants were evaluated for aphid damage 3 wk after inoculation using a

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modified 0-4 half step rating scale described by Mensah et al. (2008). The aphid resistance score was determined as the mean of the rated plants in each line for each replication. An aphid damage index (DI) for each line was used as an indicator of aphid resistance, ranging from 0 (no damage) to 100 (most severe damage (Mensah et al., 2005). DI was calculated based on the following formula: DI = $\sum$ (scale value x no. of plants in each category) / (4 x total no. of plants) x 100 (Zhang et al., 2009a).

DNA extraction and SSR marker genotyping

In the field trial of 2009, the unopened trifoliate from each individual plant of each line ($F_{4:5}$ mapping population) and their parents were bulk harvested for the genomic DNA extraction. The CTAB (Hexadecyltrimethyl ammonium bromide) described by Kisha et al., (1997) was used to extract the genomic DNA. The concentration was determined with a ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington Delaware).

The genomic DNA from each RIL line and parent was amplified by PCR protocol with SSR markers described by Cregan and Quigley (1997) on a MJ Tetrad^{TM} thermal cycler (MJ Research Waltham, MA). The sequence information of SSR primers was provided by Dr. Perry Cregan (USDA-ARS, Beltsville, Maryland). A total of 1056 SSR primers were used to screen for the polymorphism between PI 567585A and 'Skylla'. Bulked segregant analysis (Michelmore et al., 1991) was used to accelerate the

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identification of the aphid resistance locus. Ten resistant lines with the lowest DI scores were selected and bulked into a resistance pool for analysis. The resistant bulk and parental DNA samples were genotyped with polymorphic markers. Priority was placed on the polymorphic SSRs on chromosomes 7, 13, and 16 (LG M, F, and J) with coverage of a marker at every 10 cM because these LGs were linked to aphid resistance in other soybean accessions.

The PCR products were separated on 6% non-denaturing polyacrylamide gels with a DASG-400-50 electrophoresis unit (C.B.S. Scientific Co., Del Mar, CA) as described by Wang et al., (2003). The ethidium bromide stained gels were visualized and photographed under UV light. For polymorphic SSR markers, the PCR products of each line in the mapping population were scored as 'a' (only the band of the resistant parent present), 'b' (only band of susceptible parent present) or 'h' (bands from both parents present).

Statistical and QTL analysis

The DI data from the field trial was analyzed by the analysis of variance (ANOVA) with the GLM procedure of SAS V9.1. The broad-sense heritability of DI was estimated according to the method described by Fehr (1987). The SSR genotyping data and the aphid resistance phenotyping data of $F_{4:5}$ RIL lines were analyzed to construct a linkage map with Join-Map 3.0 by using the Kosambi function and a LOD score of 3.0 (Van

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Ooijen and Voorrips, 2001). At each locus of potential aphid resistance, the segregation ratio of alleles was determined by X^2 goodness of fit to detect if the locus met the expected 7:2:7 ratio with a significance threshold of $P = 0.05$. Composite interval mapping (CIM) was performed to detect aphid resistance loci by using QTL Cartographer V2. with a standard model Zmapqtl 6 (Wang et al., 2008). In order to control the genetic background, the forward and backward regression method was applied to select markers other than the interval being tested as cofactors (Zeng, 1994). A window size of 10 cM was chosen and the target markers interval distance was at 2 cM for CIM. The empirical LOD at 5% probability level was determined by a 1,000- permutation test (Churchill and Doerge, 1994). The linkage map and the aphid resistance loci were visualized by MapChart (Voorrips, 2002).

Resistance locus validation

A validation population of 162 $F_{3:4}$ lines (070016) was derived from the cross between PI 567585A and IA 2070 by single seed descent. IA 2070 is an aphid-susceptible soybean cultivar. In the summer of 2009, the validation population and its parents were evaluated for aphid resistance in a field trial similar to the mapping population with two replicates. Ninety four RILs were randomly selected as a subset population from the validation population. The genomic DNA of these 94 RILs was extracted by method described above. Polymorphic markers within the potential regions

containing the aphid resistance locus were genotyped for the validation population. Linkage map construction and genetic mapping analysis were carried out in the same way as for the mapping population.

RESULTS

Phenotypic data analysis for mapping and validation populations

The phenotypic data for aphid damage index of mapping and validation populations, and parents in the field trial were shown in Table 3.1. Resistant parent PI 567585A had significantly ($P<0.05$) lower DI than susceptible parents 'Skylla' and IA2070, which were heavily infested by soybean aphids. The broad-sense heritabilities for aphid resistance were 0.96 and 0.89 for population 070082 and 070016 in the field trial, respectively (Table 3.1). This indicates that substantial variation exists among RILs within both mapping and validation populations. The DI for the two populations showed discontinuous variation and approximate bimodal distribution with a ratio of 1:1, confirming that aphid resistance is controlled by one single gene (Figure 3.1) (Liu et al., unpublished data).

Genetic mapping of aphid resistance

A total of 313 SSR markers were polymorphic between PI 567585A and 'Skylla'. Analysis of the bulked resistant lines from the 070082-2 population indicated that the

SSR markers Satt622 and Satt215 on chromosome 16 (LG J) were associated with aphid resistance. These two SSR markers were genotyped for the entire 070082 RIL population, and their associations with aphid resistance were confirmed. Five other polymorphic SSR markers within ± 30 cM of Satt622 and Satt215 were genotyped for the entire mapping population. The segregation of all markers except Satt674 fit a 7:2:7 (homozygous SSR allele of the resistant parent: heterozygous SSR alleles from both resistant and susceptible parents: homozygous SSR allele of the susceptible parent) segregation ratio ($P>0.05$) at F_4 generation (Table 3.2).

A linkage group was constructed by analyzing these seven markers with Join-Map. The marker order was highly consistent with the consensus map (Song et al., 2004), although the spanning distance was 70.2 cM, about 7.3 cM larger than the corresponding map distance of 62.9 cM (Figure 3.2). The aphid resistance gene was identified in the interval between Satt674 and Sct_065 in both greenhouse and field trials (Figure 3.2 and Table 3.3). The major phenotypic variation of aphid resistance contributed by the PI 567585A gene in the greenhouse and field trials was 93.1% and 90.1%, respectively. The additive effect of this resistance gene was also determined for the mapping population in both trials. The PI 567585A resistance allele decreased the soybean aphid DI value by 32.35 and 30.50 in the greenhouse and field trials, respectively (Table 3.3). In addition, the average DI value was calculated for each genotype class of Sct_065, and analyzed by ANOVA. The heterozygous class showed an intermediate level of resistance to soybean

aphid between resistant and susceptible classes, which was not significantly different from the average of the two homozygous classes (Table 3.4). These results indicated that aphid resistance in PI 567585A is controlled by a single co-dominant gene with additive effect, which is consistent with a previous genetic inheritance study of PI 567585A (Liu et al., unpublished data).

Validation of aphid resistance gene

Seven SSR markers linked to the resistance locus on LG J in the mapping population were genotyped for the validation population (070016). The segregation ratio of each marker fit the 7:2:7 ratio (Table 3.2). These seven markers were used to construct a linkage map, which was similar to the consensus map, except for the inverted order of Satt654 and Sct_065 (Figure 3.2). A single aphid resistance gene was identified in the interval between Satt654 and Sct_065 in the QTL analysis using the CIM analysis. This gene is located at a position of 8 cM above Sct_065, which is the same as the QTL detected in the mapping population. Moreover, the resistance gene identified in the validation population explained 85.6% of the phenotypic variation in the field trial (Table 3.3). Thus, analysis of the validation population confirmed the location of the aphid resistance locus identified in the mapping population.

DISCUSSION

The increased genetic distance in two mapping populations for LG J was due to three possible reasons, when compared to the consensus map: 1) the parents used for our SSR map are more distantly related and expected to have a lower recombination rate; 2) the average distance is larger among the markers, which are closely linked to the resistance gene; 3) Join-Map utilizes two-point detection unlike the MapMaker that uses three-point detection approach. However, MapMaker cannot be set to accept data from F4-derived families. The aphid resistance in PI 567585A was controlled by a single co-dominant gene with additive effect, that mapped between Satt674 and Sct_065 on Chromosome 16 (LG J). In a previous study, the resistance gene in PI 567543C was also found to be a single co-dominant gene possessing additive effects. This gene was located in the same general region as the aphid resistance gene *Rag3* in PI 567543C (Zhang et al., 2009b). Further fine mapping of *Rag3* and *Rag3-1* with more SNP markers will be considered to identify the relationship between them, either closely-linked or multiple allelism within the same locus.

The discovery of a common mapping location for two aphid resistance genes was not unexpected because the two PIs were both collected from Shandong province, China (Chen et al., 2007). This kind of genetic allelism also exists in other soybean aphid resistant accessions. For example, *Rag1* in 'Dowling' and *Rag* in 'Jackson' were mapped to the same position on LG M (Li et al., 2007). The resistance gene in PI 200538 was mapped to the same region as the aphid resistance gene *Rag2* in PI 234550 on soybean

LG F (Mian et al., 2008; Hill et al., 2009). Moreover, a QTL conferring resistance to brown stem (caused by *Phialophora gregata*) was mapped to the same region in five different PIs, which all originated from central China (Klos et al., 2005). So data suggested that the resistance gene (*Rag3_1*) in PI 567585A is not a new aphid resistance gene, but may be a resistance source in addition to *Rag3* of PI 567543C.

The mapping of soybean aphid resistance to LG J is interesting because several resistance genes, such as powdery mildew resistance locus (*Rmd*), corn earworm (CEW) resistance genes (CEW6-2 and CEW 7-4), brown stem rot (BSR) resistance gene, *Phytophthora* resistance *Rps2*, soybean cyst nematode (SCN) race-2 and race-3 resistance genes, sudden death syndrome resistance genes (SDS), and soybean rust resistance genes (*Rpp2*) (Grant et al., 2009), has been localized to the LG. In addition, five classes of disease resistance gene analogs (RGAs) were extensively clustered on chromosome 16 (LG J), including RGA1, RGA2, RGA3, RGA5, and RGA6 (Kanazin et al., 1996). Some previous studies showed the presence of genes in the same region conferring resistance to several diseases may explain the correlation between a variety of disease resistance. For example, the similar gene location for BSR and powdery mildew resistance (*Rmd*) were suggested to explain the positive association between these resistance traits (Lewers et al., 1999). Among these resistance loci, the *Rag3-1* region only overlapped with the two CEW resistance QTLs and SDS resistance locus (Sanitchon et al., 2004). Previous studies on CEW and aphid resistance showed that the two traits were inherited separately. So it is

suggested that aphid resistance gene *Rag3* or *Rag3-1* and the CEW QTLs may not occupy the same locus on chromosome 16. However, it is unknown if the association of aphid resistance and SDS resistance exists, whether the underlying resistance genes for both traits are separated, close-linked or pleiotrophic.

At least three soybean aphid biotypes have been discovered: the Illinois, Ohio and Michigan biotypes (Kim et al., 2008; Dr. Dechun Wang, unpublished data). The aphid resistance genes *Rag1* and *Rag2* did not provide resistance in plants that were infested with the aphid collected from Michigan in 2008 and 2009 (Dr. Dechun Wang, unpublished data). However, *Rag3* in PI 567543C conferred a broad resistance to aphid isolates from Ohio and Michigan (Zhang et al., 2009a). The gene in PI 567585A provided an additional source of *Rag3*, resistance to Michigan aphid isolates in the field trial in 2008 and 2009. This aphid resistance gene locus and the linked molecular markers will be useful for developing new aphid-resistant soybean cultivar.

Table 3.1 Damage index of soybean aphid in the field in the summer of 2009 for the parents: PI 567585A, Skylla, and IA2070; 158 $F_{4:5}$ RILs derived from 070082-2 validation population (PI 567585A x 'Skylla'); and 162 $F_{3:4}$ RILs derived from 070016-2 mapping population (PI 567585A x IA2070).

Population ID	Parents			RILs population		
	PI 567585A	IA 2070	'Skylla	Mean±SE*	Range	H^2**
70082	16.7a	-	87.5b	58.1±8.55	12.5~87.5	95.50%
70016	12.5a	73.2b	-	39.7±12.55	8.3~87.5	88.70%

Means followed by different letters within the same row are significantly different at $P<0.05$

$DI^+=\sum$ (scale value x no. of plants in each category) / (4 x total no. of plants) x 100.

SE*= standard error

H^2**=broad sense heritability

Table 3.2 X^2 test of segregation ratio for the aphid resistance gene (Rag3-1) and seven SSR markers among 158 $F_{4:5}$ RILs from the PI 567585A x 'Skylla' mapping population and 94 $F_{3:4}$ RILs from the PI 567585A x IA 2070 validation population.

Population ID	Locus	Number of $F_{3:4}$ RILs in each category				$X^2_{7:2:7}$	*P*
		a*	b*	h*	-*		
070082	Satt674	77	7	74	0	9.472	0.0088
	Sct_065	75	22	62	0	1.474	0.4785
	Satt406	70	17	69	1	0.374	0.8296
	Satt654	75	18	62	3	1.358	0.5072
	Satt622	74	27	57	0	5.132	0.0768
	Satt215	68	26	64	0	2.376	0.3048
	Satt431	76	12	70	0	3.736	0.1544
	Rag3-1	70	19	69	0	0.040	0.9803
070016	Satt674	36	6	52	0	6.328	0.0423
	Sct_065	40	15	39	0	1.040	0.5947
	Satt406	51	2	41	0	10.462	0.0053
	Satt654	40	11	43	0	0.164	0.9212
	Satt622	46	9	39	0	1.331	0.5139
	Satt215	34	13	47	0	2.207	0.3318
	Satt431	44	7	43	0	2.207	0.3318
	Rag3-1	40	12	42	0	0.055	0.9730

a*=homozygous SSR allele of the resistant parent, PI 567598B

b*=homozygous SSR allele of the susceptible parent, 'Skylla' or IA 2070

h*=heterozygous SSR alleles from both resistant and susceptible parents

-*=missing band for SSR alleles

Table 3.3 Summary for aphid resistance loci detected in the mapping population and the validation population with aphid DI data using the CIM method

Population	Trial	LG/ Chr*	Peak Pos.(cM)**	Flanking markers+	LOD	R^{2++}	$a^{\ddagger}$
070082	Greenhouse	J/16	15.5	Satt674~Sct_065	21.66	93.1	32.35
	Field Cage	J/16	16.0	Satt674~Sct_065	15.66	90.1	30.50
070016	Field Cage	J/16	20.0	Satt654~Sct_065	28.17	85.6	26.25

LG/Chr*=linkage group/chromsome

Peak Pos.(cM)**=QTL peak position is expressed in cM

Flanking markers+=Markers flanking the peak position or the marker at the peak position

R^{2++}=Percentage of phenotypic variation explained by a QTL

$a^{\ddagger}$=Additive effect. The positive value implies that the PI 567598B allele decreases the DI

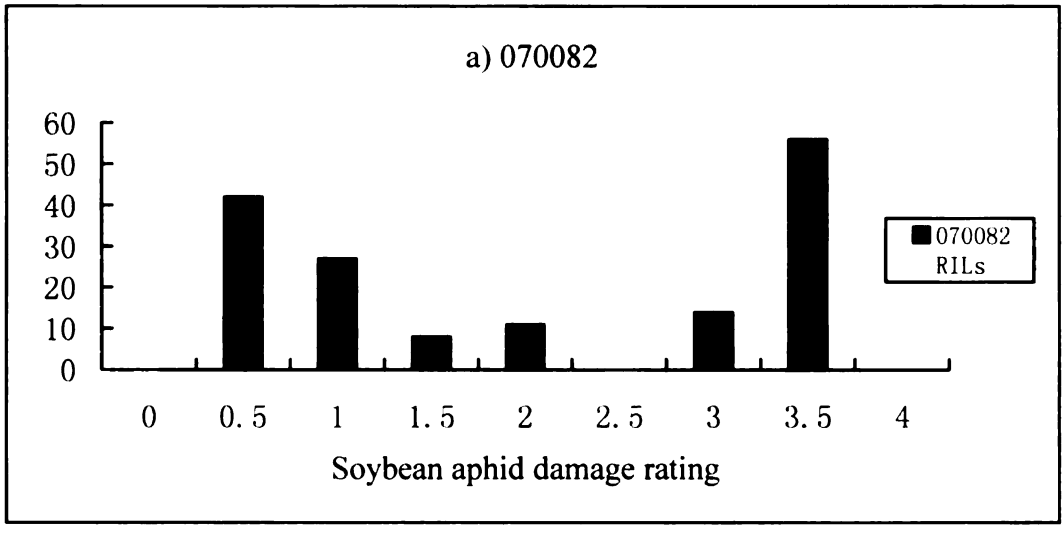
Table 3.4 Average aphid DI for different genotypes of marker Sct_065 in the field trial for mapping and validation populations

Population	PI 567585A Type (a^1)	Heterozygous Type (b^2)	Skylla / IA 2070 Type (h^3)	Average of PI 567585A Skylla / IA 2070 type
070082	39.75a	53.75b	81.25c	60.50b
070016	22.50a	42.50b	67.50c	45.00b

a^1=homozygous SSR allele of the resistant parent, PI 567598B

b^2=homozygous SSR allele of the susceptible parent, 'Skylla' or IA 2070

h^3=heterozygous SSR alleles from both resistant and susceptible parents



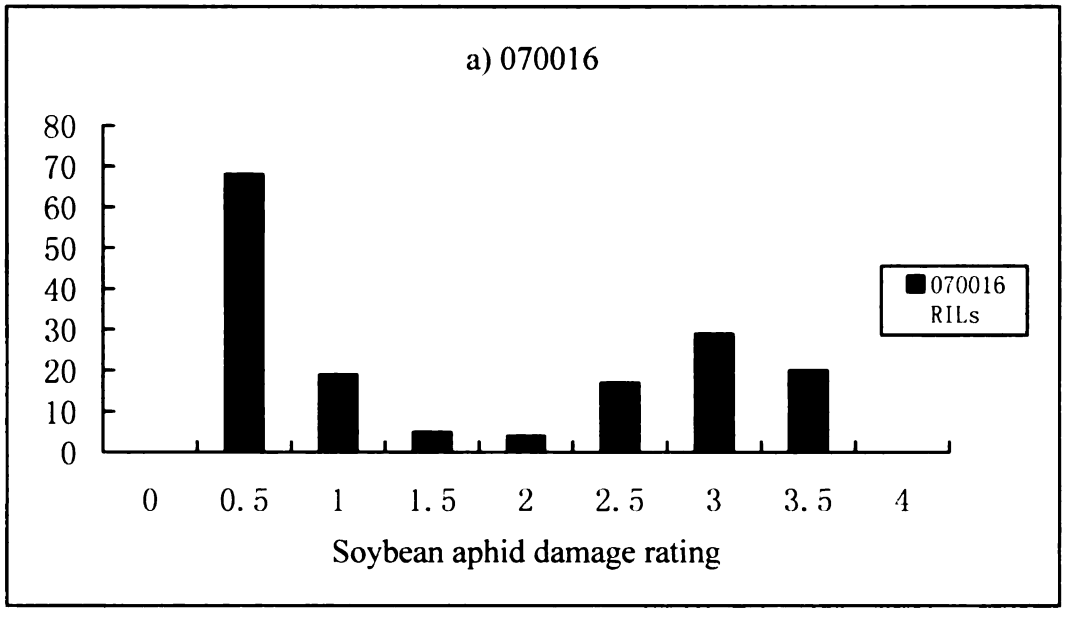


Figure 3.1 Distribution of DI scores in RIL populations: a) 070082 $F_{4:5}$ RILs validation population; b) 070016 $F_{3:4}$ RILs mapping population.

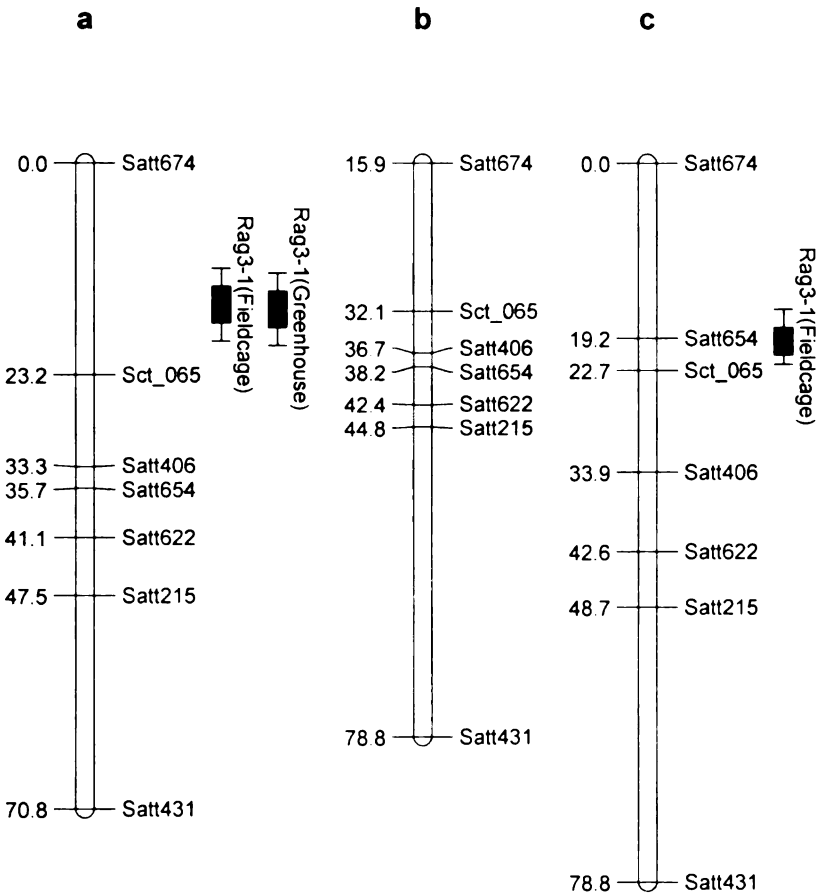


Figure 3.2 Linkage maps showing the locations of the soybean aphid resistance genes from PI 567585A that were mapped on soybean linkage group J. (a) Aphid resistance gene position in the mapping population for greenhouse and field trials. (b) The relevant segment of the soybean LG according to the integrated soybean map of Song et al., (2004). (c) Aphid resistance gene position in the validation population.

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CHAPTER 4

IDENTIFICATION OF APHID RESISTANCE GENES IN SOYBEAN USING MODIFIED NESTED ASSOCIATION MAPPING (MNAM)

ABSTRACT

The soybean aphid (*Aphis glycines*) has become an important pest of soybean [*Glycine max* (L.) Merr.] in the U.S. since 2000. PI 567598B was found to possess antibiosis resistance to the soybean aphid. In this study, a modified nested association mapping (MNAM) approach was used to locate resistance genes in PI 567598B on the integrated soybean linkage map. PI 567598B was crossed with 10 different susceptible cultivars to construct 10 recombinant inbred lines (RILs) populations, where only resistant progenies were selected in each population for MNAM. We expected that the genomic regions containing the aphid resistant genes from PI567598B were present in the most resistant progenies. Chi-square test was used to discover the significant association between aphid resistance and single sequence repeat (SSR) markers. False discovery rate and Bonferroni correction were applied to control the type I error rate. Genomic regions on linkage groups F, G, J and M were found associated with soybean aphid resistance in MNAM. Linkage analysis of a population of 94 $BC_1F_{4:5}$ RILs derived from PI 567598B and a $F_{4:5}$ RIL population derived from E06902 (elite advance breeding line developed from PI567598B) were used to confirm the MNAM results. The results of linkage analysis showed that genomic regions on the linkage groups F, J, and N were associated

with aphid resistance. Ultimately, we showed that MNAM was efficient for the discovery of aphid resistance genes in soybean breeding and germplasm improvement.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is a leading crop worldwide, providing an important source of oil and protein. In 2008, the soybean production area was 30.19 million hectares in the US, and the production represented 38% (80 million metric tons) of the world's total soybean production (USDA-National Agricultural Statistics Service, 2008). Insect pests, including the soybean aphid (*Aphis glycines* Matsumura), are the major constraints to the production and economic yield of soybean. Soybean aphids feed directly on soybean aboveground biomass and transmit several viruses, causing 8-25% yield loss in Michigan (DiFonzo and Hines, 2002). Native to Asia, the soybean aphid was detected in the U.S. in July 2000. Since then, it has spread to at least 21 states in the US and 3 provinces in Canada, becoming one of the most destructive pests of soybean in North America (Chen et al., 2007).

Insecticides are currently the only effective method to control soybean aphid. However, utilization of host resistance could provide a more practical method of pest control without releasing pesticides into the environment and increasing production costs (Sun et al., 2000). Host resistance is classified as tolerance, antixenosis, or antibiosis (Painter 1951). Tolerance is defined as host plant resistance that restricts an infestation

without yield loss. Antixenosis is present when insect pests show non-preference for a specific host plant. Antibiosis reduces insect abundance by disrupting the life cycle of the insect during feeding, decreasing plant damage. Antibiosis and antixenosis have been studied extensively in soybean aphid resistance research. Choice and nonchoice tests have been used to distinguish between these two resistance mechanisms. Recently, certain soybean plant introduction lines were found to possess antibiosis resistance in soybean germplasm: 'Dowling', 'Jackson' (Hill et al., 2004), PI 567541B, PI 567598B (Mensah et al., 2005); 'K1639', 'Pioneer 95B97', PI 230977 (Diaz-Montano et al., 2006; Hesler et al., 2007); PI 59099 (Hesler et al., 2007); PI 243540 (Kang et al., 2008), PI 567543C (Zhang et al., 2009b), and PI 567585A (Dr. Dechun Wang, unpublished paper). Classic genetic studies indicate that aphid resistance in 'Dowling', 'Jackson', PI 200538, and PI 243540 is controlled by a single dominant gene (Hill et al., 2006a, 2006b; Mian et al., 2008a; Hill et al., 2009), and that the aphid resistance in PI 567541B and PI 567598B is controlled by two recessive genes (Mensah et al., 2008; Zhang et al., 2009a). Recently, one major co-dominant gene was shown to control the aphid resistance in PI 567585A was identified (Liu et al., unpublished data).

In complex agricultural traits such as aphid resistance, many quantitative trait loci (QTL) contribute to phenotypic variation, each with a small effect and influenced by environmental factors. Thus, QTL mapping was developed to identify QTLs associated

with a desirable phenotype. Linkage analysis and association mapping are the two widely used approaches for exploring QTLs underlying quantitative traits (Zhu et al., 2008).

The linkage analysis was widely used in mapping soybean aphid resistance. This genetic mapping can detect marker allele-trait associations within a structured pedigree, such as an F_2, a backcross or a $F_{4:5}$ RIL population. Six aphid resistance genes have been located on F, J and M linkage groups (LG) by linkage analysis: *Rag1* in 'Dowling', *Rag* in 'Jackson' (Li et al., 2007), *Rag2* in PI 243540 and PI 200538 (Mian et al., 2008b; Hill et al., 2009), *Rag3* in PI 567543C (Zhang et al., 2009b), and *rag1_3* and *rag4* in PI 567541B (Zhang et al., 2009a). However, the limitation of linkage analysis is low-resolution mapping in a specific population, leading to inconsistent results occurring among different populations. Therefore, association mapping is regarded as an essential supplement to linkage analysis because it allows high-resolution and genome-wide QTL scanning (Zhu et al., 2008). Nested association mapping (NAM) was developed for maize (*Zea mays* L.) to dissect the genetic inheritance of complex quantitative traits by combining the benefits of linkage analysis and association mapping (Yu et al., 2008). As the common parent, inbred line B73 was crossed with other 25 founder parents. A total of 5000 RILs derived from these 25 founder populations were genotyped by common parent specific (CPS) markers. By using computer simulation, NAM detected the historical recombination in populations and identified the relatively narrow gene regions underlying complex traits (Yu et al., 2008). However, discovering resistance genes by applying

NAM directly is impractical. First, during artificial selection, susceptible lines will be discarded after each generation. It is also not necessary to retain all RIL populations for genotyping. Second, plant breeders have to consider the conflicts between rapid cultivar replacement and the time spent to create numerous founder populations. The cost of genotyping is another restriction for most plant breeding programs.

In this study, we developed a modified nested association mapping (MNAM) design which is more suitable for detecting the aphid resistance genes in soybean breeding and germplasm improvement. PI 567598B serves as a common aphid resistance parent, which was crossed with 10 founder susceptible parents. Ten F_2 populations were advanced to F_4 generation through single seed descent (SSD). Unlike NAM, we only selected and analyzed resistant progenies in each $F_{4:5}$ RIL founder populations. Because of the high Type I error in association mapping, MNAM also uses Bonferroni and false discovery rate (FDR) controlling procedures to adjust the genome-wide false positives (Yu et al., 2008). In summary, the objectives of this study were to: 1) identify the genes responsible for aphid resistance in soybean PI 567598B by using MNAM; 2) confirm the resistance genes by using traditional linkage analysis.

MATERIALS AND METHODS

Primary and secondary populations for MNAM

The eleven founder soybean accessions were PI 567598B, Titan, A00-711003, A00-711020, A02-381100, E00003, IA2064, IA2070, IA2072, SDA00R-039-42, and Skylla. The common resistant parent, PI 567598B, was crossed with the other 10 founder accessions, followed by selfing, to generate 10 segregating F_2 populations. Out of each population, RILs were derived through single seed descent. Individual RILs of each $F_{4:5}$ population were evaluated for soybean aphid resistance in greenhouse and field during 2006. A total of 85 resistant RILs were obtained (Table 4.1). Among them, 41 $F_{4:5}$ resistant RILs were derived from the cross of PI 567598B and Titan, which was called the primary population. In the secondary population, 44 resistant RILs were developed from the cross of PI 567598B with the other nine founder parents (Dr. Dechun Wang, unpublished data).

DNA extraction, PCR and gel system

For primary, secondary, and confirmation populations, the unexpanded trifoliates of each line were harvested and pooled for genomic DNA isolation. The DNA was isolated by the CTAB method (Kisha et al., 1997) and the DNA concentration was detected by a ND-1000 Spectrophotometer (NanoDrop Technologies, Inc; Wilmington, DE). SSR markers (Song et al., 2004) were used to amplify the genomic DNA according to the PCR protocol described by Cregan and Quigley (1997) using a MJ Tetrad^{TM} thermal cycler (MJ Research, Waltham, MA). PCR products were detected on 6% non-denaturing

polyacrylamide gels by using a DASG-400-50 electrophoresis system (C.B.S. Scientific Co., Del Mar, CA) as described by Wang et al., (2003). Gels stained with ethidium bromide were photographed and scored under UV light.

Identification of QTLs by MNAM

MNAM included the following steps:

1) Screen the polymorphism of 1056 SSR markers in parents PI 567598B and Titan in the primary population along the whole genome. The SSR primer sequences were obtained from Dr. Perry Cregan at USDA-ARS, Beltsville, MD.
2) Genotype 41 RILs in the primary population using the polymorphic SSR markers of approximate 20 cM interval coverage in each linkage group.
3) Use the Chi-square test to detect SSR markers significantly associated with soybean aphid resistance in the primary population, and apply Bonferroni and FDR methods to control for false positive results.
4) Select SSRs that were consecutively and significantly associated with resistance over an approximate distance of 30 cM. The selected SSRs were assigned to several potential regions that potentially contribute to the aphid resistance in the primary population.
5) Genotype 11 parents of the primary and secondary populations using saturated polymorphic SSR markers within the potential aphid resistance regions. The SSRs

with rare specific banding pattern for PI 567598B were called common-parent-specific (CPS) SSRs. The selection criterion for these PI 567598B-rare SSRs were set to be segregating in >8 populations.

6) Genotype CPS SSRs in the secondary populations. Those CPS SSRs significantly associated with resistance in both primary and secondary populations were defined as consistent-common-parent-specific (CCPS) SSRs.

Statistical analysis

1) Chi-square test

For the inheritance of each SSR marker locus, the segregation of the banding patterns in the primary and secondary populations in MNAM were tested to fit the expected ratio of 1:1 (resistant parent: susceptible parent) by Chi-square tests. The amplified SSR bands were scored and classified: homozygous for the banding pattern of PI 567598B (a); heterozygous for the banding patterns of PI 567598B and susceptible parent (h); or homozygous for the banding patterns of the susceptible parent (b). The observed number of resistant RILs (m) that inherited the banding pattern from PI 567598B was calculated as the sum of a and h. The p-values of Chi-square tests were calculated for 1 degree of freedom. In each individual Chi-square test, the SSR marker locus was considered significantly associated with aphid resistance when $p \leq 0.01$ and the banding patterns were skewed toward the resistant parent.

2) Bonferroni corrections and FDR-controlling procedures

Because MNAM in the primary population was a procedure of multiple hypotheses tests, p-values obtained for each marker locus by the Chi-square test were subjected to Bonferroni and FDR correction procedures (Benjamini and Yekutieli, 2005), in order to reduce false discoveries. In the Bonferroni correction, $\alpha \leq 0.01$ was fixed for entire set of N Chi-square tests. For each Chi-square test, the SSR was regarded as significantly associated with aphid resistance if the $p \leq \alpha/N$. In the FDR-controlling procedure, the QVALUE software was used to estimate the q-values for the list of *p*-value resulting from the multiple Chi-square tests (Storey, 2002). Q-value was controlled at the level of 0.01.

Validation of QTLs by linkage mapping

1) Confirmation populations

The following two populations were constructed to map aphid resistance genes in PI 567598B, and test the efficiency and accuracy of MNAM.

First, a $BCF_{4:5}$ population was composed of 94 RILs, which were derived from a cross between PI 567598B and Titan, that was backcrossed with Titan. Genotyping was carried out on this population with the CCPS SSRs discovered in MNAM. Considering the monomorphism resulting from backcrossing, polymorphic markers between two

parents were genotyped within the potential aphid resistance gene regions around CCPS SSRs.

Second, an $F_{4:5}$ RIL population (070063) was developed from the cross between E06902 and IA2070. E06902 is an elite advanced breeding line derived from the original PI 567598B resistance source that possessed resistance levels similar to PI 567598B in field evaluations during 2006 (Dr. Dechun Wang, unpublished data). E06902 was crossed to the susceptible soybean accession IA 2070 in 2007, followed by selfing, to generate segregating F_2 population. SSD was applied to reach F_4 generation. A total of 118 $F_{4:5}$ RILs were obtained. Considering the monomorphism due to inbreeding practice, polymorphic markers between two parents were added within the potential aphid resistance gene regions around CCPS SSRs.

2) Evaluation of soybean aphid resistance

For the $BCF_{4:5}$ population, aphid resistance was evaluated in the greenhouse in spring 2008 without any replication and in the field in 2008 summer with two replicates. Phenotypic data were collected by evaluating aphid resistance in the field in the summer of 2009 without any replication in the $F_{4:5}$ RIL population (070063). The greenhouse trial was conducted in the Plant Science Greenhouse at Michigan State University (MSU) in East Lansing Michigan. Eight seeds per line or parent were planted in a plastic pot, which is 210mm in diameter and 125mm deep. The two parents and the mapping population

were set on the bench without replication in a completely randomized design (CRD). The temperature was maintained at 26/15°C day/night with 14-h supplemental lighting provided by sodium vapor lamps. The field evaluation of soybean aphid resistance was carried out in a 12.2 x 18.3m aphid- and predator- proof cage (Redwood Empire Awning Co., Santa Risa, CA) on the Agronomy Farm of MSU. The parental plants were planted randomly in the field, 5.1 cm apart. Each RIL was planted in a single row plot, 60cm long with a row spacing of 60cm. The average number of plants per line was around 10 with most plots having at least 12 plants. A similar CRD was used to arrange each population and its parents in the field plots.

In both greenhouse and field trials, each plant was inoculated at the V2 stage with two wingless aphids. The $BC_1F_{4:5}$ population and parental plants were evaluated for aphid damage without replication in greenhouse in 2008 spring, with two replications in field in 2008 summer. A single aphid clone was collected from a naturally infested field at the MSU Agronomy Farm in summer 2007, and maintained in an isolation chamber in the greenhouse for the inoculation of plants in the greenhouse trial in 2008 spring. The soybean aphids used for inoculation in the field trial were collected from a naturally infested field on the MSU Agronomy Farm during 2008 summer. The $BC_1F_{4:5}$ population and parental plants were evaluated for aphid damage the 3rd and 4th week after inoculation using a modified 0-4 half step rating scale described by Mensah et al., (2008). The $F_{4:5}$ RIL population (070063) and parental plants were evaluated for aphid damage

without replication in field in 2009 summer. Same aphid infestation and damage rating methods were used as described before.

The aphid resistance score was determined as the mean of the rated plants in each line for each replication. An aphid damage index (DI) for each line was used as an indicator of aphid resistance, ranging from 0 (no damage) to 100 (most severe damage (Mensah et al., 2005). DI was calculated based on the following formula: DI = $\sum$ (scale value x no. of plants in each category) / (4 x total no. of plants) x 100 (Zhang et al., 2009a).

3) Statistics for linkage analysis

Linkage analysis was performed for these two populations with Map Manager QTXb20 (Manly et al., 2001). The linkage maps were constructed using JoinMap and MapChart (Van Ooijen, 2001; Voorrips, 2002). Then linkage groups were assigned to specific chromosomes according to the soybean consensus map (Song et al., 2004). Simple interval mapping (SIM) and composite interval mapping (CIM) were applied to locate QTLs for aphid resistance with the use of QTL Cartographer V2.5 (Zeng, 1994; Wang et al., 2008). The QTL results for the confirmation populations obtained by linkage analysis were compared to the results from MNAM to test whether these QTLs are co-located at the same positions along chromosomes.

RESULTS

1) Identification of QTL regions in the primary population

Among 1056 SSR markers, 311 polymorphisms were identified between the parents in the primary population. In order to accelerate the identification of potential regions associated with aphid resistance, 112 polymorphic SSR markers at an average distance of 22 cM were used to genotype 41 resistant RILs in the primary population. The amplified SSR bands were scored, and the m was defined as the observed number of resistant RILs that inherited the banding pattern from PI 567598B in the primary population. Figure 4.1 shows an example of a banding pattern amplified by primer satt406 in the primary population. A total of 38 resistant RILs showed a banding pattern inherited from PI 567598B. P-value was 4.6E-08 based on the Chi-square test, indicating that satt406 was significantly associated with aphid.

For the Bonferroni correction, the alpha value of the entire set of 112 comparisons was 0.01. For each comparison, the alpha value equaled to 8.9E-05 (0.01/112). In the FDR controlling procedure, the q-values were calculated according to the p-values of Chi-square tests. A total of 18 consecutive SSR markers were significantly associated with aphid resistance over an approximate distance of 30 cM. These 18 SSR markers were distributed on five linkage groups: B_2, F, G, J and M (Table 4.2). Five potential regions were defined as associated with aphid resistance in the primary population: from 66 to 95 cM on B_2 linkage group (Region B2); from 16 to 130 cM on F linkage group

(Region F); from 22 to 59 cM on G linkage group (Region G); from 15 to 52 cM on J linkage group (Region J); and from 38 to 67 cM on M linkage group (Region M).

2) Identification of CPS SSRs

Eleven parents of the primary and secondary population were genotyped using saturated polymorphic SSR markers within the five potential regions. PI 567598B showed specific banding patterns at 18 SSR marker loci, unlike the ten susceptible parents. These SSR marker loci, whose banding patterns differed from those of the susceptible parents were defined as CPS SSRs (Figure 4.2 and Table 4.3), distributing over four linkage groups: F, G, J, and M. No CPS SSRs were located in the potential regions on LG B_2.

Identification of CCPS SSRs

The eighteen CPS SSRs were tested for the association with aphid resistance in the primary and secondary population. The p-values were calculated for each CPS SSR locus in each population (Table 4.3). Fourteen CPS SSRs were identified as CCPS SSRs, which meant they were consistently and significantly associated with aphid resistance in both populations. The CCPS SSRs were located on three linkage groups: F, G, and J.

Validation of QTLs in two confirmation populations

Seven polymorphic SSRs within the potential aphid resistance regions on LG J were genotyped for 94 lines in the $BC_1F_{4:5}$ population. The QTL was located between Satt285

and Satt380, which were mapped in the interval of 9.5 and 27.1 cM on the soybean consensus mapping. The QTL explained 69.2%~91.1% of the phenotypic variance for both 3rd and 4th week screening in the greenhouse and field trials (Table 4.4). The soybean aphid resistance QTL identified in MNAM and linkage mapping was located at the similar position along the linkage group J (Figure 4.3). The marker Sat_304 on LG N was found to be linked with aphid resistance gene, explaining 4.1%~8.1% of phenotypic variation for 3rd week in either greenhouse or field trials.

Six polymorphic SSRs within the potential aphid resistance regions on linkage group J were genotyped on 112 lines in a $F_{4:5}$ RIL population (070063). The soybean aphid resistance QTL identified in MNAM and linkage mapping was located at the similar position along the linkage group J (Figure 4.3). The QTL was located between Sct_065 and Satt596, which are mapped in the interval of 16.1 and 23.8 cM on the soybean consensus mapping. The identified QTL explained 53.4% of the phenotypic variation for 3rd week in the field trial. The markers Satt334 (LG F) and Sat_304 (LG N) identified as linked with aphid resistance loci, explaining 11% and 18% of phenotypic variation for 3rd week in field trial (Table 4.4).

Band pattern analysis of markers linked to QTLs for aphid resistance

Five aphid resistance germplasm, accession PI 567543C, PI567541B, PI 567598B, PI 567585A, and 'Dowling', together with two susceptible accessions were genotyped using markers Satt030 (LG F), Satt522 (LG F), Satt622 (LG J), Satt529 (LG J), Satt463

(LG M), Sat_304 (LG N), which were tightly linked with the potential QTLs identified in this study. On LG J, the band patterns of the PCR products from PI 567598B were as same as PI 567585A for both of the two markers, but different from Dowling. The PI 567598B and PI 567541B accessions share the same band pattern for marker Satt46 on LG M, whereas PI 567543C and PI 567585A share a different band pattern.

DISCISSION

1) Modified nested association mapping

The resistant genotypes present a pattern of gene identity by descent (IBD), which is underlying the patterns of observed phenotypes. Genotypes of progenies or relatives are similar because they share genes that are IBD, inherited from a common ancestor within the defined pedigree. Several studies have been carried out to infer the IBD information of QTL by using flanking markers, including nested association mapping (Charlier et al., 1996). NAM was first proposed and implemented in maize; in order to dissect complex traits at the gene level by using designed multiple mapping populations from linkage analysis. The genetic architecture of quantitative traits, for flowering time and northern leaf blight (NLB) has been studied using NAM (Buckler et al., 2009; Chung et al., 2008; Poland et al., 2009).

In the MNAM experiment, PI 567598B is the common parent, whose resistance gene(s) can be followed in the progenies through flanking CPS SSRs. The CPS SSR

allowed the prediction of inheritance of chromosome segments in progenies among populations, rather than tested of bi-allelic contrasts across each bi-parental population (Yu et al., 2008). However, all RIL in each population were retained and analyzed for the genetic dissection of quantitative traits in NAM. In this study, unlike these quantitative traits analyzed in NAM, soybean aphid resistance has been discovered to be controlled by oligogenes in a number of resistant PIs, either as one or two dominant/recessive genes. So selection was used to increase the efficiency in MNAM, where only resistant RILs derived from founder populations were selected and genotyped in MNAM.

2) Resolution of MNAM

Association mapping exploit the historical recombination events at the population level. The resolution of association mapping relies on the density of molecular markers, and the linkage disequilibrium (LD) between an array of linked markers and the functional mutations responsible for trait variation. Generally, LD decays at a much greater distance in self-pollinated crops than in cross-pollinated species. For example, the LD decayed within 0.4~10 kb in maize depending on the gene length (Flint-Garcia et al., 2003). In cultivated soybean, LD extended from 90 to 574 kb because of increased self-fertilization during domestication (Hyten et al., 2007). Thus, if LD decays within a long distance, the mapping resolution will be low, and a relatively small number of markers are required in soybean association mapping. The goal of MNAM is to quickly

the aphid resistance gene location/s on the soybean consensus map. In consideration of the cost and necessity of genotyping, relatively low-resolution SSR markers were used to localize the QTL regions. SNP markers would be added to the potential regions to pinpoint the genetic variation at the gene level in future fine mapping study.

3) Association mapping and linkage mapping

Comparison of locations of reported QTL showed that more significant QTLs identified using association mapping were located within the previously reported QTL regions. In association mapping of yellow pigment in durum wheat germplasm, 48% of the significant markers identified in AM were associated with QTLs found through linkage analysis (Reimer et al., 2008). Due to the combined information across all families, the NAM analysis identified nearly twice as many significant QTLs compared with individual family linkage analysis. A total of 29 QTLs were identified that explained 64% of the ASI (anthesis-silking interval) variation, meanwhile 36 and 39 QTLs contributed to the 89% of the total variance for DA (days to anthesis) and DS (days to silking). The QTLs identified in NAM were concordantly located within six major QTL regions previously mapped in meta-analysis of maize flowering date (Buckler et al.,, 2009). As another quantitative disease resistance trait, NLB resistance was investigated for genetic dissection by NAM. A total of 21 QTLs were detected in this study, but new QTLs were also detected. Most of these QTLs co-localized with previous identified

disease resistance loci for NLB. Furthermore, *qEt8.06* (*qEt* for quantitative resistance to *Exserohilum turcicum*) was consistently identified as the largest-effect QTL across all populations, and one QTL on chromosome 8 significantly contributing to resistance (Chung et al., 2008).

In this study, the aphid resistance gene region on LG J (chromosome 16) was detected in MNAM, and then confirmed by linkage analysis. This QTL region explained 53.4~91.1% of aphid resistance variation either in greenhouse and field trials. In MNAM, additional two resistance regions were found on LG F and N (chromosome 13 and 03). The QTL on LG F was confirmed only in population 070063, not in the $BC_1F_{4:5}$ population, which may be due to different genetic background of these two populations. Similar aphid resistance regions on LG F were identified in other soybean aphid resistance sources, PI 200538 and PI 243540. The QTL explaining 4.1%~18% of phenotypic variation on LG N was identified in both populations for phenotypic data evaluated in 3rd week. The Sat_304 marker was significantly associated with aphid resistance in the first step of MNAM, but no consecutive SSR markers significantly associated with aphid resistance around Sat_304 were detected. Thus, the QTL region on LG N was not studied further and regarded as potential aphid resistance QTL region in MNAM.

4) Prediction of molecular function of aphid resistance QTLs in PI 567598B

In the past decade, resistance to insects has been identified in various plant species. A series of *R* genes have been mapped, and molecular markers linked to these loci have been identified. These identified genes confer resistance to Russian wheat aphid, Hessian fly, and *Mayetiola destructor* (Kaloshian, 2004) in wheat. In this study, the resistance genes on LG J and N of soybean genome were identified together mainly in the 3rd week. The genome sequence search showed that a cluster of leucine-rich-repeat (LRR), toll and interleukin-1 receptor (TIR), and nucleotide binding site (NBS) genes were located within the region between Satt285 and Satt380. It is not surprising that an array of R genes exist within a resistance locus conferring disease resistance. For example, three candidate genes were identified within the NLB resistance locus $qNLB8.06_{DK888}$, including two tandem protein kinase(PK)-like genes and one protein phosphatase(PP)-like gene (Chung et al., 2008). Thus, fine mapping and cloning of candidate genes is required to identify their real functional roles of individual members.

Moreover, several ERF, MYB, WRKY transcription factors (TF) were located on LG N closely linked to Sat_304. These results are reasonable because the induction of plant defense by insect feeding is regulated by several signaling pathways, including salicylic acid (SA)-, jasmonic acid (JA)- regulation pathways. Li et al. (2008) demonstrated that aphid feeding on soybean induced expression of NBS-LRR, Myb family TF, and genes associated with both SA and JA mediated response pathways. In their study, Gm-r1070-4664, a potential MYB family transcription factor, is one of the top five

constitutively higher expressed genes in aphid resistance in cv. Dowling. By genome sequence search, the closely linked marker to Gm-r1070-4664 was Sat_304 located on LG N (Chromosome 03), near the aphid resistance locus found in this study. The TFs could be potentially involved in the early basal resistance stage, before the initiation of numerous expression of R genes. Thus, it may be reasonable to collect phenotypic data in the 3rd week to capture/detect more QTL regions conferring aphid resistance in soybean.

5) The relationship among different soybean aphid resistant sources

Understanding the allelic relationship among different resistance sources can be used to determine the breeding strategy to control the most effective alleles R gene pyramid. PI 567541B, PI 567543C, PI 567598B, and PI 567585A all originated from Shandong province, China. In this study, the major QTL of PI 567598B was coincidently located in a similar genomic region on LG J as the resistance genes in PI 567543C and PI 567585A in both MNAM and linkage analysis. PI 567598B share the same SSR amplification bands as PI 567585A, but different from PI 567543C. On LG F, the band patterns did not show similarity between PI 567598B and PI 567541B for Satt030, suggesting that there is no resistance locus on the upper region of LG F in PI 567598B. Also, unpublished data showed that the segregation ratio of F_2 progenies derived from a cross between PI 567598B and PI 567541B was 3:1, indicating that resistance genes on LG F and J both contribute to the resistance. All above information indicated that the

resistance loci in PI 567598B, PI 567585A and PI 567543C are either allelic at the same locus or tightly linked genes on LG J.

PI 567598B and PI 567541B shared the same banding patterns for Satt463 on LG M, and this resistance locus was detected in MNAM (Figure 4.4). However, this locus was not detected in the confirmation population using linkage analysis. Moreover, the segregation ratio suggested the locus on LG M not involving in the resistance in PI 567598B. It is possible that the resistance loci in these two PIs are located within the small region, where no recombination event occurs, or maybe the resistance loci on LG M, such as *Rag1*, were already overcome due to the evolution of resistant aphid biotypes in Michigan (Dr. Dechun Wang, unpublished data). As mentioned above, the resistance is generally determined by one or few members in the *R* genes cluster. Several populations derived from crosses among these resistance sources will be used to determine whether the aphid resistance is conferred by same R gene member or different members in individual PI accessions.

6) MNAM and breeding practice

In this study, MNAM was developed to investigate the genetic basis of traits controlled by oligogenes that could offer several advantages for the plant breeding and genetic research community. First, the susceptible lines are generally discarded in each generation each year. Facing the rapid cultivar replacement in market place, the critical

point in marker-assisted selection or molecular plant breeding is the timely application of molecular markers. Thus only aphid resistant RILs in each population were used in MNAM, which means the population built for breeding purposes can also be used in MNAM for genetic studies, without the intent to construct bi-parental population for QTL mapping. Eventually, plant breeders conducting selection to achieve breeding goals, can generate genetic research at the same time. The cost of maintaining and genotyping whole RIL population in each founder population restricts many breeding research labs using NAM for genetic studies. In MNAM, partial founder populations are retained and genotypes, which decrease the labor time and expense. However, MNAM is not suitable for quantitative traits, whose phenotypic data show a normal distribution, as selection from extreme tails will be problematic. Thus MNAM favors qualitative traits or traits controlled by oligogenes.

Table 4.1 List of primary and secondary populations subjected to MNAM

Population name	Female (Susceptible parent)	Male (Common resistant parent)	Number of $F_{4:5}$ resistant RILs
Primary population	Titan	PI 567598B	41
Secondary population	A00-711003	PI 567598B	5
	A00-711020	PI 567598B	7
	A02-381100	PI 567598B	2
	E00003	PI 567598B	3
	IA2064	PI 567598B	3
	IA2070	PI 567598B	5
	IA2072	PI 567598B	2
	SDA00R-039-42	PI 567598B	8
	Skylla	PI 567598B	9

Table 4.2 Consecutive SSR markers significantly associated with aphid resistance on five linkage groups: B_2, F, G, J and M.

SSR marker name	Linkage group	Linkage Group Position	m^A	p-value	q-value
Sat_355	B_2	66.24	36	1.29E-06	1.26E-05
Satt474	B_2	75.35	35	5.93E-06	4.75E-06
Satt063	B_2	93.49	34	2.48E-05	1.69E-05
Satt252	F	16.08	35	5.93E-06	4.75E-06
Satt663	F	56.17	36	1.29E-06	1.26E-06
Sat_120	F	75.97	36	1.29E-06	1.26E-06
Satt490	F	97.97	36	1.29E-06	1.26E-06
Satt522	F	119.19	35	5.93E-06	4.75E-06
Sat_090	F	130.64	35	5.93E-06	4.75E-06
Satt235	G	21.89	36	1.29E-06	1.26E-06
Satt340	G	48.54	35	5.93E-06	4.75E-06
Satt594	G	52.94	35	5.93E-06	4.75E-06
Satt287	J	15.69	34	2.48E-05	1.69E-05
Satt285	J	25.51	32	3.28E-04	1.99E-04
Satt406	J	38.19	38	4.6E-08	2.48E-05
Sat_366	J	52.84	32	3.28E-04	1.99E-04
Satt435	M	38.94	36	1.29E-06	1.26E-06
Sat_226	M	65.79	35	5.93E-06	4.75E-06

m: calcaulated as the observed number of resistant RILs that inherited the banding pattern from PI 567598B in the primary population.

Table 4.3 CCPS SSR markers significantly associated with aphid resistance on three linkage groups: F, G, and M.

SSR marker	Linkage group	Linkage Group Position	Observed number of RILs inherited the banding pattern from the common parent PI 567598B (m)		p-value	
			Primary population (41)	Secondary population (44)	Primary population	Secondary population
satt252	F	16.08	35	27	5.93E-06	0.132
satt423	F	20.56	34	18	1.26E-05	0.228
sat_240	F	25.58	35	27	5.93E-06	0.132
satt663	F	56.17	35	30	5.93E-06	0.016
Satt334	F	76.41	34	38	1.26E-05	1.41E-06
satt522	F	119.19	34	33	1.26E-05	6.30E-04
sat_308	G	43.09	35	30	5.93E-06	0.016
satt115	G	43.78	33	27	9.45E-05	0.132
satt594	G	52.94	35	34	5.93E-06	1.90E-04
satt406	J	38.19	38	39	4.60E-08	2.96E-07
satt596	J	39.64	37	41	2.55E-07	1.01E-08
sat_151	J	41.35	33	33	9.45E-05	6.30E-04
satt529	J	41.90	38	39	4.60E-08	2.96E-07
satt622	J	42.25	38	36	4.60E-08	2.43E-05
satt380	J	43.01	33	38	9.45E-05	1.41E-06
sat_255	J	43.85	38	36	4.60E-08	2.43E-05
satt215	J	44.08	35	34	5.93E-06	1.90E-04
sct_001	J	44.68	36	38	1.29E-06	1.41E-06

Table 4.4 Summary for aphid resistance locus detected in mapping population and the validation population with aphid DI data using the CIM/SIM method

Population	Trial	Rep/ week	LG/Chr[a]	Peak Pos.(cM)[b] /Consensus Pos.[c]	Flanking markers[d]	LOD[e]	R^{2f}	a[g]
$BC_1F_{4:5}$	Greenhouse	1/3	J/16	25.41	Satt285~Satt406	10.17	69.2%	0.86
	Greenhouse	1/3	N/3	77.10	Sat_304	-	8.0%	0.20
	Greenhouse	1/4	J/16	22.41	Satt285~Satt406	8.98	89.2%	0.81
	Field Cage	1/3	J/16	25.91	Satt285~Satt406	13.39	79.1%	0.88
	Field Cage	1/3	N/3	77.10	Sat_304	-	4.2%	0.15
	Field Cage	2/3	J/16	24.91	Satt285~Satt406	16.36	78.6%	0.89
	Field Cage	2/3	N/3	77.10	Sat_304	-	8.1%	0.26
	Field Cage	1/4	J/16	25.91	Satt406~Satt380	12.87	84.6%	1.09
	Field Cage	2/4	J/16	25.91	Satt285~Satt406	22.87	91.1%	1.09
070063	Field Cage	1/3	J/16	7.14	Satt414~Satt280	6.31	53.4%	1.02
	Field Cage	1/3	F/13	78.06	Satt334	-	11%	0.40
	Field Cage	1/3	N/3	77.10	Sat_304	-	18%	0.52

[a] LG/Chr=linkage group/chromsome

[b] QTL peak position is expressed in cM

[c] consensus position is expressed in cM

[d] Markers flanking the peak position or the marker at the peak position

[e] The LOD threshold is 6.8 and 4.6 for $BC_1F_{4:5}$ and 070063populations, respectively

[f] Percentage of phenotypic variation explained by a QTL

[g] Additive effect. The positive value implies that the PI 567598B allele decreases the phenotypic value

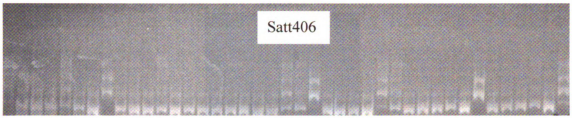


Figure 4.1 SSR amplification banding patterns of 41 resistant RILs in the primary population using primer satt406. Upper and lower bands were the amplification banding pattern for Titan and PI 567598B using SSR primer satt406, respectively.

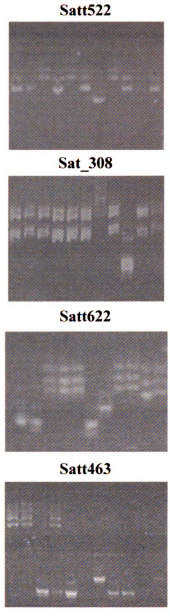


Figure 4.2 Banding patterns of PCR products of the 11 parents in the primary and secondary populations using SSR marker Satt522 (LG F), Sat_308 (LG G), Satt622 (LG J), and Satt463 (M). The order of the 11 parents is: A00-711003(1), A00-711020(2), A02-381100(3), E00003(4), IA2064(5), RR Titan(6), PI 567598B(7), IA2070(8), IA2072(9), SDX00R-039-42(10), Skylla(11).

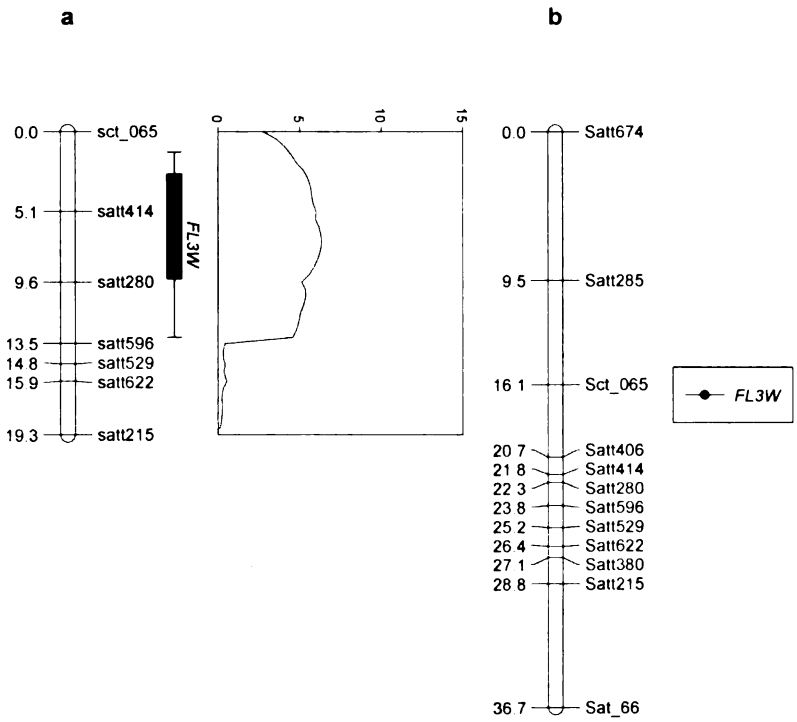
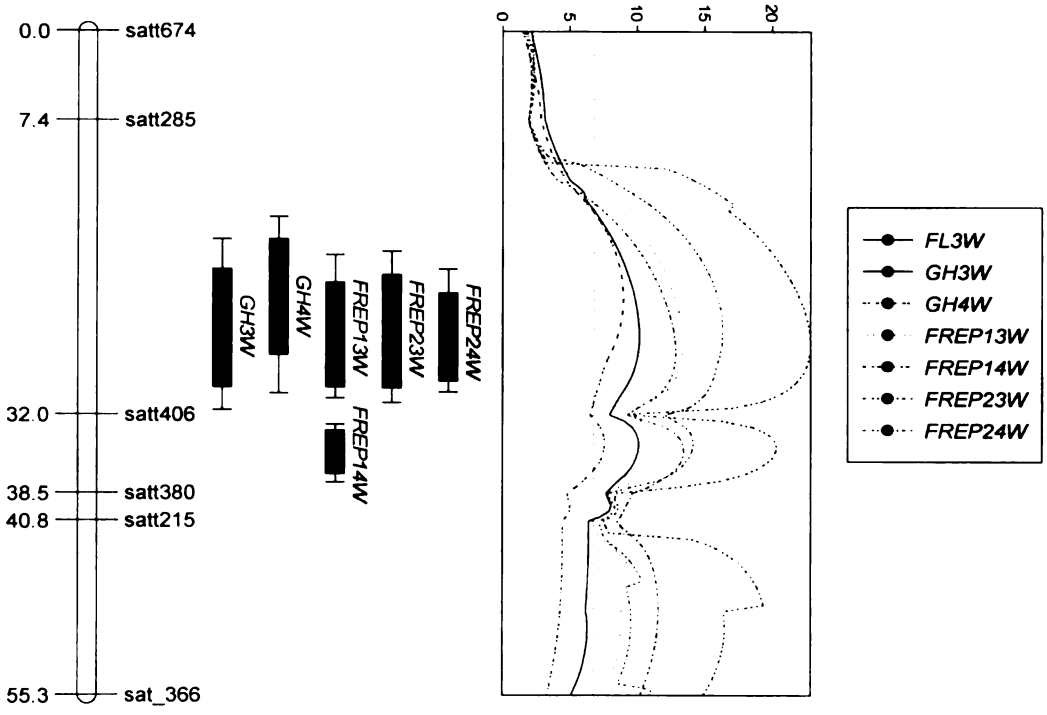


Figure 4.3 Locations of soybean aphid resistance locus on LG J using composite interval mapping method. a map shows the identified resistance locus in $BC_1F_{4:5}$ Population for 3-week and 4-week rating in either greenhouse or field trials: greenhouse 3-week (*GH3W*), greenhouse 4-week (*GH4W*), replication 1 for 3-week rating in field trial (*FREP13W*), replication 1 for 4-week rating in field trial (*FREP14W*), replication 2 for 3-week rating in field trial (*FREP23W*), replication 2 for 4-week rating in field trial (*FREP24W*). b map is the soybean consensus map for linkage group J. In c map, the filled black bar represents the locus for the 3-week rating in the field cage trial (F*L3W*) for $F_{4:5}$ RIL population (070063). In a and c map, the LOD threshold is 6.8 and 4.6 for $BC_1F_{4:5}$ and 070063populations, respectively.

Figure 4.3 (Cont'd)

c



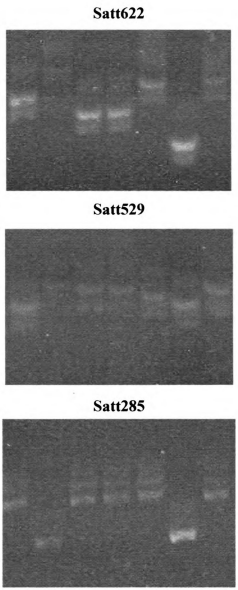


Figure 4.4 PCR products amplified by SSR markers Satt622 (LG J), and Satt529 (LG J), for PI 567543C (1), PI 567541B (2), PI 567598B (3), PI 567585A (4), Skylla (5), Titan (6), Dowling (7).

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APPENDIX

Table A1 0-4 Half step scale for soybean aphid damage (Mensah et al., 2008)

Half Step Scale	Soybean Aphid Damage
0	No aphids, health and normal plants
0.5	Less than 10 aphids, no colony formed, health and normal plants
1	11-100 aphids/plant, still normal and health plants
1.5	101-150 aphids/plant, mostly located on the young leaves
2	151-300 aphids/plant, located both on the young leaves and stems
2.5	301-500 aphids/plant, aphids dispersed on leaves and stems
3	501-800 aphids/plant, curly and shiny leaves, young leaves and stems covered by aphids
3.5	More than 800 aphids/plant, stunted plants, curled and yellow leaves, but no sooty mold and few cast skins
4	More than 800 aphids/plant, stunted plants, curled and yellow leaves, sooty mold and cast skins

Table A2 The aphid resistance scores for Skylla, IA2070, PI 567585A, and F_1 generations derived from 070082-1 and 070016-1 populations.

Genetic ID	Genetic Name	Individual No.	Rating
7P76	PI 567585A	1	1.0
7P76	PI 567585A	2	0.5
7P76	PI 567585A	3	1.0
7P76	PI 567585A	4	0.5
7P76	PI 567585A	5	0.5
7P76	PI 567585A	6	1.0
7P76	PI 567585A	7	0.5
7P76	PI 567585A	8	1.0
7P02	Skylla	1	3.5
7P02	Skylla	2	3.5
7P02	Skylla	3	3.5
7P02	Skylla	4	3.5
7P02	Skylla	5	3.5
7P02	Skylla	6	3.5
7P02	Skylla	7	3.5
7P02	Skylla	8	3.5
7P02	Skylla	9	3.5
7P02	Skylla	10	3.5
7P38	IA2070	1	3.5
7P38	IA2070	2	3.5
7P38	IA2070	3	3.0
7P38	IA2070	4	3.5
7P38	IA2070	5	3.5
7P38	IA2070	6	3.5
7P38	IA2070	7	3.5
7P38	IA2070	8	3.0
7P38	IA2070	9	3.5
7P38	IA2070	10	3.5
7P38	IA2070	11	3.0
	(Skylla x PI 567585A) F_1	1	2.5
	(Skylla x PI 567585A) F_1	2	2.5
	(Skylla x PI 567585A) F_1	3	2.5
	(Skylla x PI 567585A) F_1	4	2.0
	(Skylla x PI 567585A) F_1	5	2.5
	(IA2070 x PI 567585A) F_1	1	-
	(IA2070 x PI 567585A) F_1	2	-
	(IA2070 x PI 567585A) F_1	3	-
	(IA2070 x PI 567585A) F_1	4	-
	(IA2070 x PI 567585A) F_1	5	-

Table A3 Aphid resistance scores for 070016-1 F_2 population

070016-1 F_2 population		070016-1 F_2 population		070016-1 F_2 population	
F_2 line No.	Rating	F_2 line No.	Rating	F_2 line No.	Rating
1	2	31	2	61	3
2	2.5	32	2	62	3
3	2	33	2.5	63	2
4	1.5	34	3.5	64	3.5
5	3	35	2	65	2
6	3	36	1	66	1
7	2	37	2.5	67	0.5
8	1	38	3	68	1
9	2	39	1.5	69	2.5
10	2	40	1	70	1
11	2	41	1.5	71	2
12	2.5	42	2	72	2
13	0.5	43	1	73	3
14	2.5	44	2	74	2
15	1	45	1.5	75	-
16	2	46	1	76	1
17	1	47	2	77	1
18	2	48	2	78	1
19	3	49	1.5	79	2
20	1.5	50	2	80	3
21	1.5	51	1.5	81	1
22	1	52	1.5	82	2.5
23	1	53	1.5	83	2
24	2.5	54	1.5	84	3.5
25	2	55	1.5	85	3
26	2	56	2	86	2
27	1	57	1	87	2
28	2	58	3	88	3
29	2	59	2.5		
30	2	60	1		

Table A4 Aphid resistance scores for 070082-1 F_2 population

070082-1 F_2 population		070082-1 F_2 population		070082-1 F_2 population	
F_2 line No.	Rating	F_2 line No.	Rating	F_2 line No.	Rating
1	3	31	1.5	61	3
2	1	32	2	62	0.5
3	1.5	33	1	63	2
4	1.5	34	1.5	64	1.5
5	2	35	0.5	65	1.5
6	2	36	1.5	66	3
7	1	37	1	67	3
8	1	38	1.5	68	3
9	1.5	39	0.5	69	2.5
10	3	40	0.5	70	1
11	-	41	0.5	71	3
12	2.5	42	0.5	72	1.5
13	1.5	43	1	73	3
14	1	44	1.5	74	3
15	1.5	45	2.5	75	1.5
16	1.5	46	0.5	76	2
17	3	47	2	77	3
18	1	48	0.5	78	3
19	2	49	1.5	79	1.5
20	1	50	0.5	80	2.5
21	2	51	0.5	81	1
22	1.5	52	1.5	82	3.5
23	1	53	0.5	83	2
24	2.5	54	2	84	0.5
25	1.5	55	2	85	1
26	1.5	56	2.5	86	1.5
27	1.5	57	1.5	87	2
28	2	58	1.5	88	1
29	2	59	1	89	0.5
30	1.5	60	1.5	90	1.5

Table A4 (Cont'd)

070082-1 F_2 population		070082-1 F_2 population		070082-1 F_2 population	
F_2 line No.	Rating	F_2 line No.	Rating	F_2 line No.	Rating
91	1.5	121	0.5	151	1
92	1.5	122	1.5	152	1.5
93	2	123	2	153	2
94	1	124	2	154	1.5
95	1.5	125	2	155	2
96	1.5	126	2	156	1.5
97	1	127	1	157	2
98	1.5	128	1	158	1
99	1.5	129	0.5	159	1.5
100	1	130	1.5	160	0.5
101	1	131	2	161	1.5
102	0.5	132	1.5	162	2
103	1.5	133	1.5	163	1.5
104	0.5	134	1	164	2
105	1.5	135	0.5	165	1.5
106	1	136	2	166	3
107	2	137	1	167	2
108	2.5	138	1.5	168	1
109	1.5	139	2	169	3
110	2	140	2.5	170	1.5
111	0.5	141	3.5	171	3
112	0.5	142	3	172	0.5
113	1	143	3	173	1.5
114	1.5	144	3	174	1.5
115	1.5	145	3	175	1.5
116	1	146	1	176	1.5
117	0.5	147	1.5	177	1.5
118	2	148	1.5	178	1.5
119	2	149	3	179	1
120	1	150	3	180	-

Table A4 (Cont'd)

070082-1 F_2 population		070082-1 F_2 population	
F_2 line No.	Rating	F_2 line No.	Rating
181	1.5	203	1
182	1.5	204	3
183	2	205	3
184	1	206	3.5
185	3	207	2.5
186	1	208	3
187	1.5	209	1.5
188	0.5	210	1.5
189	2	211	2.5
190	1.5	212	1
191	0.5	213	3.5
192	1.5	214	2
193	3	215	3.5
194	3.5	216	3
195	3	217	3
196	3	218	3.5
197	1.5	219	1.5
198	1.5	220	1.5
199	3	221	3.5
200	1.5	222	3.5
201	3	223	3.5
202	3	224	3.5

Table A5 Progeny test for $F_{2:3}$ families derived from 070016-1

F_2 individual tested in 2008		$F_{2:3}$ progenies in 2009										
Line	WK3	1	2	3	4	5	6	7	8	9	10	11
1	2	3	1.5	3	1.5	3	2	2	2	3.5	2.5	2.5
2	3	3	2	3.5	3.5	2.5						
3	2	1.5	1	1.5	1.5	1	1.5	1.5	1			
4	2	3.5	1.5	1.5	3.5	3.5	1.5					
5	2	3.5	2.5	3.5	3	3.5	3.5	3.5	3.5			
6	3	1	2	2	3	1.5	2	2.5	1.5			
7	2	1	1.5	1.5	1.5	1	1					
8	1	2.5	2	2.5	1	1.5	2	3.5	1.5			
9	2	3.5	3.5	3	3.5	3.5						
11	2	2	1.5	2	1.5	2.5	3.5	1.5	2			
12	2.5	2.5	1	2	3.5	3.5	3	3.5	3.5	1	2	
13	0.5	1	1	1	1.5	1	1.5	1				
15	1	3.5	1	1.5	2	1.5	1.5	3	1.5	1		
19	3	1	3	2	3	2.5	1.5	1.5	1			
20	1.5	3.5	3.5	3.5	3.5							
21	1.5	1.5	3	3.5	1.5	3.5						
22	1	0.5	1	0.5	0.5	1	1	1				
26	2	1	1	3.5	1	3	1.5	2.5	3.5			
27	1	3.5	3	3.5	1.5	1.5						
30	2	3.5	3.5	3								
31	2	1	1	1	1	1						
32	2	2	1.5	2	3	3.5	1.5	3.5	2	1		
35	2	3.5	3.5	3.5	3.5	1.5	1.5					
36	1	2	1.5	1.5	2.5	2	2	1.5	3	2	1	
37	2.5	1.5	1.5	2.5	3.5	2	3.5	2	1.5	1.5	1.5	3.5
41	1.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5		
44	2	1.5	3	3.5	1	3	1.5	1.5	3.5	1.5	2	
47	2	0.5	1	1	0	0.5	0.5	0.5	0.5	0.5		
48	2	1	0.5	1	2	3.5	1.5	1				
49	1.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5				
50	2	3.5	2	3.5	3.5	3.5	1	1	1.5			
51	1.5	1	1	1	1.5	1.5	2	1	0.5	1.5	1.5	
52	1.5	0.5	0.5	0.5	1.5	1.5	0.5	0.5	1	1	0.5	0.5
55	1.5	1	1	0.5	0.5	1	1	1	1	1		
56	2	1	3	0.5	0.5	1	0.5	1				
58	3	1.5	1.5	1	2.5	3.5	1.5	1.5	1			
59	2.5	1	0.5	0.5	0.5	0.5	1	1.5				
60	1	1.5	3.5	3.5	1.5	2.5						
61	3	3.5	3.5	3.5	3.5							

Table A5 (Cont'd)

F_2 individual tested in 2008	$F_{2:3}$ progenies in 2009											
Line	WK3	1	2	3	4	5	6	7	8	9	10	11
63	2	3.5	3.5	3.5	3.5	3.5						
64	3.5	3.5	3.5	3.5	3.5	3.5	3.5					
65	2	1.5	0.5	0.5	0.5	1	1	0.5				
66	1	1	1	3.5	2	1	3.5	1	1.5	0.5	1	
67	0.5	3.5	3.5	3.5	3	1.5						
68	1	0.5	0.5	0.5	0.5							
69	2.5	0.5	0.5	0.5	0.5	0.5	0.5					
72	2	1.5	3.5	3.5	2	3.5	0.5	0.5				
73	2.5	0.5	3.5	0.5	0.5	1	0.5	1.5				
78	1	2	0.5	1	3	3.5	1.5	1				
79	2	3.5	3.5	3.5	3.5							
80	3	0.5	3	3	3	3	3	1	3.5			
81	1	0.5	1	0.5	0.5	0.5	0.5					
82	2.5	0.5	0.5	0.5	0.5	0.5						
83	2	1	1	1.5	1.5	2	1	1.5				
84	3.5	3.5	3.5	3.5	3.5	3.5	3.5					
85	3	3.5	3.5	3.5	3.5	3.5						
86	2	2	1	1	1	2	2	3.5	2			
88	3	3.5	3.5	3.5	3.5							

Table A6 Progeny test for $F_{2:3}$ families derived from 070082-1

F_2 individual tested in 2008	$F_{2:3}$ progenies in 2009											
Line	WK3	1	2	3	4	5	6	7	8	9	10	11
1	3	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	
2	1	2	2	1	2	2	1	1.5	3.5	1		
3	1.5	3.5	3.5	3.5	3.5	3.5	2	2	3.5			
4	1.5	3	3	2	1	1	2	3.5	2.5	1.5	1	
6	2	3.5	3.5	3.5	3.5	3.5	3.5					
10	3	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5			
15	1.5	3.5	1	1	3	1	2.5	1	1	3		
16	1.5	1.5	1	1.5	1.5	1.5	1.5	1.5	1	1.5		
18	1	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5			
19	2	3.5	3.5	3.5	3.5	3.5	3.5	3.5				
20	1	3.5	2.5	2	2	2	2.5					
21	2	3.5	3.5	3.5	3.5	2.5	3.5	1.5	1.5	1.5		
22	1	1.5	1	1	1.5	1	1	1	1.5	1		
24	2	2	2	2	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
25	1.5	3.5	3.5	1.5	3.5	1	1					
26	1.5	1	1	1	3.5	3.5						
27	1.5	1	1	1	1	2	1.5	1	1	1		
28	2	1	1	1	1							
30	1.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5			
33	1	3.5	3.5	3.5	3.5	3.5	3.5					
34	1.5	1.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5			
35	0.5	3	3	1	3	1.5	1.5	1	1	1	1	
36	1.5	2	3.5	3.5	3.5	3.5	3.5	3.5	3.5			
37	1	1	1.5	3	3	1	1	2.5	1	1		
38	1.5	1	1	1	1	1	1	1	1	1.5		
40	0.5	1										
42	0.5	1	1	1	1	1	1	1	1			
43	1	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5		
44	1.5	1	1	1	1	1						
47	2	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5		
48	0.5	1	1	1	1	1						
51	0.5	1	3.5	3.5	1.5	1	1	3.5				
53	0.5	1.5	3.5	2	1	1						
55	2	3	3	2.5								
56	2	1	1	1	1	1	1	1	1			
59	1	1.5	1	1	1	1	1	1	1	1	1	
60	1.5	2	2	2	1.5	2	1.5	2				
61	3	2.5	2	1.5	2	2	3	2	2.5	1	1.5	
63	2	1.5	1.5	1.5	2	1.5	3	2	1.5			

Table A6 (Cont'd)

F_2 individual tested in 2008		$F_{2:3}$ progenies in 2009										
Line	WK3	1	2	3	4	5	6	7	8	9	10	11
64	1.5	1	1	1	3.5	3.5	3.5					
66	3	1	1	2	2	1	2	1	1	2		
69	2.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5				
70	1	1	1	1	1	1	1	1				
71	3	3.5	1.5	3								
73	3	3.5	2	3	2.5	3.5						
74	3	3.5	3.5	3.5	3.5	3.5	3.5					
75	1.5	3	1.5	1	2	1	3.5					
76	2	3	1	1	1	1	1.5	3.5				
77	3	3.5	3.5	3.5	3.5	3.5	3.5					
78	3	2	3.5	3.5	3.5	3.5	1.5	2	1.5	1		
79	1.5	1	1	1	1	1	2.5	1	1.5			
81	1	3.5	1	2	1	1.5	1	1	1			
82	3.5	2	3	3	2	2	3.5	3.5				
83	2	1.5	1	1	1	1	1	2				
84	0.5	1	1	1	1.5	1	1	1	1	1	1.5	
85	1	1	1	1	1	1	1	1	1	1		
86	1.5	1.5	1.5	1	3.5	1	2.5	3	1	2.5	2.5	2
87	2	3.5	1	1.5	1	1	1	1	2.5	1.5	1.5	3
88	1	1.5	1	1	1	1.5	1.5	1	1	1	1	
90	1.5	1.5	1.5	1	2	2	1.5	1	3	3.5	2	
91	1.5	3.5	3.5	3.5	3.5	3.5	3.5					
93	2	3.5	3.5	3	3.5	3.5						
94	1	1	1.5	1	1	1	1	1	1	1		
95	1.5	1	1	2.5	1	3	1.5	2.5	1.5			
96	1.5	1.5	2	1.5	1.5	2	2	2	1	1		
97	1	3.5	2.5	1.5	3.5	3.5	1					
98	1.5	1	1	2	1.5	2	3.5	3.5	3.5			
100	1	3.5	1.5	1.5	1.5	2	3.5	3.5	1	1	2	1.5
101	1	1	2.5	1	1	1	3	1.5	3	3	1	
102	0.5	1.5	1	1	1	1	1.5	1	1.5			
103	1.5	3	2	3	3	3	3	3	3.5			
105	1.5	3	1	1	1	2	3	1.5	1.5			
106	1	1.5	1.5	3	3	2.5	2.5	3				
107	2	1.5	2.5	3	1.5	1.5	3.5	3.5				
108	2.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5				
110	2	1	1	1.5	1	1.5	1	1	1.5	1	1	
113	1	1.5	1	3	1.5	1.5	2	2.5	3.5	2	2	

Table A6 (Cont'd)

F_2 individual tested in 2008		$F_{2:3}$ progenies in 2009										
Line	WK3	1	2	3	4	5	6	7	8	9	10	11
114	1.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5			
115	1.5	1	1	1	1	1	1	1				
116	1	2	1	1	1	2	1	2.5				
118	2	2	1	1	2	1	3	3.5				
120	1	1	3	1	1	1	3	1	3.5	1	1	2
121	0.5	2	1	1	1	0	0.5	0.5				
123	2	2.5	3	3.5	3	3.5	3	3	3			
124	2	2	1.5	1	1	3.5	2	3	1	1	1	1.5
125	2	0.5	0.5	0.5	0.5							
126	2	1	2	1	2	0	1	1.5	2	3		
127	1	0.5	0.5	0.5	0.5	0.5	0.5					
129	0.5	0.5	1	0	3	3	1	1	1.5			
130	1.5	2	1	1	1.5	3	2	1.5				
131	2	1.5	1.5	1.5	3.5	3.5	3.5					
133	1.5	1	3	3	1	1	1	3	1			
134	1	1	1	1	2	1	1	1	1	3.5	3.5	
135	0.5	2	1.5	3	1.5	1.5	1	1				
136	2	1	1	1	1	1.5	1	1.5	1.5			
137	1	1	1	1	1	1	1	1				
138	1.5	1.5	3	3	1	1	1.5	1				
139	2	2	1.5	3	3	1	1	3	1.5			
140	2.5	3	1	2	2.5	3	2.5	3	1.5			
141	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5				
146	1	3	1.5	1.5	1	3.5	3.5	1				
147	1.5	3.5	1	1								
150	3	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5			
151	1	2	1.5	1	1	1	1	1				
157	2	1	3	1.5	1.5	2	1.5					
159	1.5	3.5	1	1.5	1	3	3	2	3.5			
161	1.5	1.5	2.5	1.5	2	1.5	2	1.5				
162	2	3.5	2.5	1.5	1.5	2.5	3	2.5				
163	1.5	1.5	1	1.5	1.5	1.5	1	1				
164	2	3.5	3.5	3.5	3.5	3.5	3.5	3.5				
165	1.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5			
167	2	3.5	3.5	1	1	2	2.5	1	0.5			
169	3	3.5	3.5	3.5	3.5	3.5						
170	1.5	0.5	0.5	0.5	0.5	0.5	0.5					
171	3	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5		
172	0.5	1	1	0.5	1.5	2	0.5					

Table A6 (Cont'd)

F_2 individual tested in 2008		$F_{2:3}$ progenies in 2009										
Line	WK3	1	2	3	4	5	6	7	8	9	10	11
173	1.5	3.5	2	1	1	2	2					
174	1.5	2	1.5	3.5	3.5	3	1	1.5	2			
175	1.5	2	3.5	3.5	3.5	3.5	3	2.5				
176	1.5	2.5	3.5	3.5	3	3	2.5					
177	1.5	3.5	1	1.5	1	3.5	1	1	0.5			
178	1.5	1	0.5	2	3.5	2	3.5	2	2			
179	1	1	0.5	0.5	0.5	3	3	0.5	0.5			
181	1.5	2.5	1	1	1	2.5	1	2	2			
182	1.5	1.5	0.5	1	1.5	1						
183	2	1.5	3.5	3.5	3	1.5	1.5	3.5				
184	1	1.5	1	1	0	0.5	0.5	1				
185	3	3.5	3.5	3.5	3.5	2.5	3	2.5				
186	1	2.5	2	1	2.5	1	3					
187	1.5	1	1.5	3.5	2	1.5	2					
189	2	0.5	0.5	0.5								
190	1.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5			
192	1.5	3.5	3.5	3.5	3.5	3.5	3.5					
193	3	3.5	3.5	3.5	3.5	3.5	3.5	3.5				
194	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5				
195	3	1	1.5	3	1.5	1	1	3				
196	3	3.5	3.5	3.5	3.5	3.5	3.5	3.5				
198	1.5	3.5	1	1	3	3	3					
199	3	1	2.5	1	3.5							
200	1.5	2.5	3	3								
201	3	3.5	3.5	3.5	3.5	3.5						
202	3	0.5	0.5	0.5	0.5	0.5						
203	1	2.5	1.5	1	1.5	2	1					
204	3	2	2	2	2	2.5						
205	3	3.5	3.5	3.5	3.5	3.5						
206	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5				
207	2.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5				
208	3	1.5	1.5	3.5	3.5	3.5	3.5					
209	1.5	1	1	1	1	1	1	1				
215	3.5	1	1.5	1	3	3.5	2.5	3				
217	3	1	2.5	3.5	3.5	2	1	3.5				
218	3.5	3.5	3.5	3.5	3.5	3.5						
219	1.5	1	1	1.5	2	3	3.5	3.5	3.5			
220	1.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5				
221	3.5	3.5	3.5	3.5	3.5	3.5						

Table A6 (Cont'd)

F_2 individual tested in 2008	$F_{2:3}$ progenies in 2009										
Line	WK3	1	2	3	4	5	6	7	8	9	10	11
222	3.5	3.5	3.5	3.5	3.5	3.5						
223	3.5	0.5	0.5	1	1	1.5	1	3.5	3.5			
224	3.5	3.5	3.5	3.5	3.5	3.5	3.5					

Table A7 RIL information for MNAM

RIL Name	Female Parent	Male parent	RIL Name	Female Parent	Male parent
ML18	RR Titan	PI567598B	ML50	RR Titan	PI567598B
ML20	RR Titan	PI567598B	ML51	RR Titan	PI567598B
ML21	RR Titan	PI567598B	ML52	RR Titan	PI567598B
ML23	RR Titan	PI567598B	ML53	RR Titan	PI567598B
ML24	RR Titan	PI567598B	ML55	RR Titan	PI567598B
ML27	RR Titan	PI567598B	ML56	RR Titan	PI567598B
ML29	RR Titan	PI567598B	ML57	RR Titan	PI567598B
ML30	RR Titan	PI567598B	ML63	RR Titan	PI567598B
ML31	RR Titan	PI567598B	ML64	RR Titan	PI567598B
ML32	RR Titan	PI567598B	ML78	RR Titan	PI567598B
ML33	RR Titan	PI567598B	ML79	RR Titan	PI567598B
ML34	RR Titan	PI567598B	ML82	RR Titan	PI567598B
ML35	RR Titan	PI567598B	ML88	RR Titan	PI567598B
ML36	RR Titan	PI567598B	ML89	RR Titan	PI567598B
ML37	RR Titan	PI567598B	ML91	RR Titan	PI567598B
ML38	RR Titan	PI567598B	ML94	RR Titan	PI567598B
ML42	RR Titan	PI567598B	ML95	RR Titan	PI567598B
ML43	RR Titan	PI567598B	ML96	RR Titan	PI567598B
ML44	RR Titan	PI567598B	ML98	RR Titan	PI567598B
ML45	RR Titan	PI567598B	ML99	RR Titan	PI567598B
ML48	RR Titan	PI567598B			

Table A7 (Cont'd)

RIL Name	Female Parent	Male parent	RIL Name	Female Parent	Male parent
CG001	A00-711003	PI 567598B	CG132	IA2070	PI 567598B
CG004	A00-711003	PI 567598B	CG133	IA2070	PI 567598B
CG008	A00-711003	PI 567598B	CG374	IA2070	PI 567598B
CG009	A00-711003	PI 567598B	CG135	IA2072	PI 567598B
CG013	A00-711003	PI 567598B	CG375	IA2072	PI 567598B
CG255	A00-711020	PI 567598B	CG120	SDX00R-039-42	PI567598B
CG257	A00-711020	PI 567598B	CG144	SDX00R-039-42	PI567598B
CG260	A00-711020	PI 567598B	CG347	SDX00R-039-42	PI567598B
CG268	A00-711020	PI 567598B	CG348	SDX00R-039-42	PI567598B
CG270	A00-711020	PI 567598B	CG350	SDX00R-039-42	PI567598B
CG272	A00-711020	PI 567598B	CG353	SDX00R-039-42	PI567598B
CG273	A00-711020	PI 567598B	CG359	SDX00R-039-42	PI567598B
CG295	A02-381100	PI 567598B	CG386	SDX00R-039-42	PI567598B
CG297	A02-381100	PI 567598B	CG199	Skylla	PI567598B
CG041	E00003	PI 567598B	CG201	Skylla	PI567598B
CG042	E00003	PI 567598B	CG202	Skylla	PI567598B
CG301	E00003	PI 567598B	CG203	Skylla	PI567598B
CG123	IA2064	PI 567598B	CG204	Skylla	PI567598B
CG125	IA2064	PI 567598B	CG207	Skylla	PI567598B
CG127	IA2064	PI 567598B	CG208	Skylla	PI567598B
CG129	IA2070	PI 567598B	CG210	Skylla	PI 567598B
CG130	IA2070	PI 567598B	CG211	Skylla	PI 567598B

Table A8 Genome-wide screening for marker trait association in primary population

LG group	Position	SSR marker	m	total MLs	P-value	q-value
H	0.5	satt666	15	26	8.58E-02	2.57E-02
H	59	sat_334	5	36	1.29E-06	1.26E-06
H	106	satt434	18	23	4.35E-01	1.03E-01
A_1	14.65	satt572	20	21	8.76E-01	1.72E-01
A_1	32.68	satt155	11	30	3.00E-03	1.32E-03
A_1	42.8	sat_356	21	20	8.76E-01	1.72E-01
A_1	64.74	satt385	17	24	2.74E-01	6.84E-02
A_1	71.39	satt545	16	25	1.60E-01	4.48E-02
A_1	93.23	satt236	14	27	4.23E-02	1.34E-02
A_2	28	satt480	12	29	7.93E-03	2.87E-03
A_2	54	satt187	7	34	2.48E-05	1.69E-05
A_2	77.7	satt341	21	20	8.76E-01	1.72E-01
A_2	90	satt377	19	22	6.39E-01	1.34E-01
A_2	128	satt209	12	29	7.93E-03	2.87E-03
A_2	159.63	satt538	22	19	6.39E-01	1.34E-01
B_1	14.32	sat_272	0	41	1.52E-10	NA
B_1	31	sat_411	12	29	7.93E-03	2.87E-03
B_1	80.89	satt332	13	28	1.91E-02	6.51E-03
B_1	96.36	satt665	18	23	4.35E-01	1.03E-01
B_1	125.74	sat_331	0	41	1.52E-10	NA
B_2	6.05	Satt577	16	25	1.60E-01	4.48E-02
B_2	27.63	satt126	11	30	3.00E-03	1.32E-03
B_2	55.2	satt168	15	26	8.58E-02	2.57E-02
B_2	66.24	sat_355	5	36	1.29E-06	1.26E-06
B_2	75.35	satt474	6	35	5.93E-06	4.75E-06
B_2	93	satt063	7	34	2.48E-05	1.69E-05
C_1	0	satt565	15	26	8.58E-02	2.57E-02
C_1	32.1	sat_337	9	32	3.28E-04	1.99E-04
C_1	65	satt578	11	30	3.00E-03	1.32E-03
C_1	73.39	satt161	17	24	2.74E-01	6.84E-02
C_1	87	sat_207	12	29	7.93E-03	2.87E-03
C_1	128	satt180	10	31	1.04E-03	5.83E-04
C_2	41	satt281	17	24	2.74E-01	6.84E-02
C_2	91	sat_246	12	29	7.93E-03	2.87E-03
C_2	117.77	satt460	12	29	7.93E-03	2.87E-03
C_2	127.67	satt316	19	22	6.39E-01	1.34E-01
C_2	145.48	satt371	20	21	8.76E-01	NA
D_{1a}	5	sat_332	11	30	3.00E-03	1.32E-03
D_{1a}	36.23	sat_353	14	27	4.23E-02	NA
D_{1a}	55.22	satt295	2	39	7.54E-09	9.95E-09
D_{1a}	77.46	satt077	7	34	2.48E-05	1.69E-05

Table A8 (Cont'd)

LG group	Position	SSR marker	m	total MI.s	P-value	q-value
D_{1a}	106.69	Satt408	0	41	1.52E-10	NA
D_{1b}	37	satt157	16	25	1.60E-01	4.48E-02
D_{1b}	46.6	satt634	13	28	1.91E-02	6.51E-03
D_{1b}	75.29	satt005	11	30	3.00E-03	1.32E-03
D_{1b}	98.7	Satt703	11	30	3.00E-03	1.32E-03
D_{1b}	118.6	satt459	14	27	4.23E-02	1.34E-02
D_{1b}	137.1	satt271	20	21	8.76E-01	1.72E-01
D_2	6	sat_296	11	30	3.00E-03	NA
D_2	29	sat_277	18	23	4.35E-01	1.03E-01
D_2	68	satt669	11	30	3.00E-03	1.32E-03
D_2	84.62	satt311	19	22	6.39E-01	1.34E-01
D_2	93.71	satt301	21	20	8.76E-01	1.72E-01
D_2	128.95	sct_137	0	41	1.52E-10	NA
E	21	satt720	20	21	8.76E-01	1.72E-01
E	41.68	satt602	0	41	1.52E-10	NA
E	56.27	satt369	12	29	7.93E-03	2.87E-03
E	64.18	sat_381	24	17	2.74E-01	6.84E-02
E	67.92	satt553	0	41	1.52E-10	NA
F	2.23	satt325	10	31	1.04E-03	5.83E-04
F	16	satt252	6	35	5.93E-06	4.75E-06
F	26	sat_240	8	33	2.48E-05	1.69E-05
F	56	satt663	5	36	1.29E-06	1.26E-06
F	75	sat_120	6	35	1.29E-06	1.26E-06
F	98	satt490	5	36	1.29E-06	1.26E-06
F	130.46	sat_090	6	35	5.93E-06	4.75E-06
G	0	satt163	19	22	6.39E-01	1.34E-01
G	21.89	satt235	5	36	1.29E-06	1.26E-06
G	48.54	satt340	8	33	5.93E-06	4.75E-06
G	59.33	satt594	6	35	5.93E-06	4.75E-06
G	76.77	satt288	12	29	7.93E-03	2.87E-03
G	96.57	satt191	19	22	6.39E-01	1.34E-01
I	27.98	satt367	24	17	2.74E-01	6.84E-02
I	46.22	satt354	19	22	6.39E-01	1.34E-01
I	75	sat_170	19	22	6.39E-01	1.34E-01
I	82.78	satt292	11	30	3.00E-03	1.32E-03
I	98	sat_155	18	23	4.35E-01	1.03E-01
I	112.7	satt440	19	22	6.39E-01	1.34E-01
J	15	satt287	14	27	2.48E-05	1.69E-05
J	25.51	satt285	12	29	3.28E-04	1.99E-04
J	39	satt406	3	38	4.60E-08	5.74E-08
J	52.71	Sat_366	9	32	3.28E-04	1.99E-04

Table A8 (Cont'd)

LG group	Position	SSR marker	m	total MLs	P-value	q-value
J	72	satt547	13	28	1.91E-02	6.51E-03
k	30.28	satt102	18	23	4.35E-01	1.03E-01
k	45.74	satt167	16	25	1.60E-01	4.48E-02
k	54.97	satt559	19	22	6.39E-01	1.34E-01
k	87	sat_243	10	31	1.04E-03	5.83E-04
L	0	satt495	0	41	1.52E-10	NA
L	19.93	satt238	11	30	3.00E-03	1.32E-03
L	34.54	satt313	19	22	6.39E-01	1.34E-01
L	56	satt156	17	24	2.74E-01	6.84E-02
L	87.42	sat_286	14	27	4.23E-02	1.34E-02
L	107	satt373	17	24	2.74E-01	6.84E-02
M	5	satt636	20	21	8.76E-01	1.72E-01
M	19	satt150	11	30	3.00E-03	1.32E-03
M	38.94	satt435	6	35	1.29E-06	1.26E-06
M	66	sat_226	6	35	5.93E-06	4.75E-06
M	80.9	satt728	17	24	2.74E-01	6.84E-02
M	95.45	satt551	14	27	4.23E-02	1.34E-02
M	112.08	Satt210	13	28	1.91E-02	6.51E-03
N	27	satt159	12	29	7.93E-03	2.87E-03
N	58	sat_033	0	41	1.52E-10	NA
N	77	sat_304	15	26	8.58E-02	2.57E-02
N	103.34	sat_125	12	29	7.93E-03	2.87E-03
O	14.17	satt500	17	24	2.74E-01	6.84E-02
O	59.49	satt241	11	30	3.00E-03	1.32E-03
O	74.05	sat_242	12	29	7.93E-03	2.14E-10
O	86.86	satt123	25	16	1.60E-01	4.48E-02
O	93.37	satt331	22	19	6.39E-01	1.34E-01
O	118.14	satt153	17	24	2.74E-01	6.84E-02
O	129.8	sat_190	11	30	3.00E-03	1.32E-03

Table A9 CCPS detected along chromosome F, G and J in MNAM

Primer Name	LG name	Position	A00-711003	A00-711020	A02-381100	E00003	IA2064	RR Titan	PI 567598B	IA2070	IA2072	SDX00R-039-42	Skylla
satt252	F	16.08	4	3	3	4	4	3	1	3	3.4	4	4
satt423	F	20.56	3	3	3	3	3	3	1	3	3	3	3
sat_240	F	25.58	4	4	4	4	4	3	1	4	3	4	4
satt663	F	56.17	3	4	4	3	4	3	1	3	3	3	3
satt334	F	76.41	3	4	3	4	3.4	4	1	3	4	4	3
satt522	F	119.19	3	3	4	3	4	3	1	4	3	3	3
sat_308	G	43.09	3	3	3	3	3	3	1	3	3	4	3
satt115	G	43.78	3	3	3	3	3	3	1	3	3	4	3
satt594	G	52.94	3	4	3	3	3	3	1	3	3	3	4
satt406	J	38.19	4	4	3	3	3	3	1	3	3	3	4
satt596	J	39.64	4	3	3	3	3	3	1	3	3	3	3
sat_151	J	41.35	3	3	4	3	4	3	1	3	3	4	4
satt529	J	41.90	4	1	4	4	4	3	1	4	4	4	1
satt622	J	42.25	3	3	4	4	4	3	1	4	5	4	6
satt380	J	43.01	4	4	4	5	5	3	1	5	6	5	4
sat_255	J	43.85	4	4	3	3	3	3	1	3	5	3	5
satt215	J	44.08	4	4	4	3	3	3	1	3	3.4	3	3
sct_001	J	44.68	3	3	4	4	4	3	1	4	3	4	3

Table A10 Aphid resistance scoring for $BC_1F_{4:5}$ population in GH and field

Lines	G/3w	G/4w	REP1w3	REP2w3	REP1w4	REP2w4
1	0.9	1.6	0.8	0.7	0.8	0.7
2	0.9	1.3	1.5	0.5	1.2	0.5
3	0.9	0.9	0.8	0.8	1.1	0.7
4	1.8	1	1.2	0.8	1.2	0.5
5	1.8	1.8	1.5	1.2	0.9	0.9
6	1.3	1.6	1.7	1.8	1.4	1.8
7	1.3	2.1	0.7	0.5	0.5	0.5
8	1.4	1.3	1.4	1.2	0.9	1.2
9	2.2	1.3	0.8	0.8	0.6	1
10	2.2	1.8	1.9	2.3	1.7	1.6
11	1.4	2.4	1.3	1.5	0.7	0.8
12	1.7	1.7	1.1	1.8	1.2	1.3
13	1.6	1.8	1.8	1.5	1.3	1.4
14	1.1	1.3	0.5	0.6	0.5	0.5
15	1.2	1.2	0.6	0.9	0.5	0.5
16	3.1	3.2	2.8	3.3	2.2	3.2
17	2.1	2.2	1.3	0.6	0.6	0.6
18	1.5	1.1	1.5	1.2	0.8	0.5
19	1.2	0.9	1	1.5	0.5	0.8
20	1.8	2.3	1.8	1.5	1.4	1.5
21	1.8	2.8	1.2	2.1	1	1.4
22	2.1	2.8	0.8	2.8	0.8	1.7
23	1.4	2.4	1.1	1.5	1.3	0.8
24	1.5	2.8	2.5	3	1.7	2.2
25	2.4	3.1	1	2.3	1.3	3.5
26	2.6	3.5	0.7	1.7	0.5	1.4
27	2.5	3.5	2	3.1	1.7	1.8
28	2.7	3.1	2.9	2.5	2.4	1.5
29	2.4	2.3	1.4	1.3	0.8	1.1
30	2.1	3.7	2.7	2.3	2.8	2.4
31	1.4	2	0.7	0.7	0.8	0.8
32	2	2.4	1.4	1.5	1.6	0.9
33	1.6	2.2	0.5	1	0.5	0.5
34	2.9	3.5	3.2	3.5	3.3	3.8
35	2.9	3.7	3.2	3.3	3.3	3.7
36	3.3	3.6	3.3	3.3	3.3	3.8
37	3.4	3.6	3.4	3.2	3.1	3.3
38	2.5	3.3	3	2	1.4	2
39	1.6	1.9	1.6	1.4	0.9	1.2
40	2.3	3.6	3	1.6	1.3	1.6
41	2.5	1.8	1.5	1.3	0.9	1

Table A10 (Cont'd)

Lines	G/3w	G/4w	REP1w3	REP2w3	REP1w4	REP2w4
42	1.3	2	0.6	0.5	0.6	0.6
43	3.3	3.5	2.2	3.3	2.7	3.3
44	2.3	3.3	3	3.3	3.6	3.5
45	1.6	1.5	1.9	1.8	0.9	1.9
46	1.4	2.3	1.5	1	0.5	0.6
47	2.6	3.4	3.5	3.4	3.7	3.6
48	2.8	3.4	3.2	3.5	3.2	3.4
49	2.4	3.2	3.4	3.3	3.1	3.3
50	1.7	1.7	1.5	2.8	1.1	1.6
51	3.5	3.5	3.2	3.3	2.2	3.3
52	3.4	3.5	3.5	2.6	3.3	2.3
53	1.6	2.4	2	1.4	1.2	1
54	1.6	2.1	3.3	1.4	2.5	0.7
55	1.1	1.4	1.1	1.8	0.8	1.1
56	3.3	3.5	2.8	2.9	3.8	3.2
57	3.3	3.5	3.5	3	3.4	3
58	1.1	2.4	1.6	1.1	0.6	0.5
59	1.4	2	1.9	0.9	0.8	0.6
60	1.5	1.3	2.1	0.8	1.1	1.1
61	3.4	4	3.3	3.3	2.5	3.3
62	1.7	2.5	1.3	3.1	0.5	1.1
63	3.1	3.5	2.7	3.3	3.3	3.4
64	2.6	3.4	2.8	3.4	2.8	3.4
65	2	3.3	2.7	3.3	2.7	3.4
66	1.4	1.9	0.6	0.5	0.6	0.5
67	2.1	2.6	0.7	2.4	0.7	1.4
68	2	2.7	0.9	0.7	0.8	0.7
69	2.4	3.5	3.5	2.6	3.6	2.4
70	1.6	1.8	1.1	1.5	0.9	1.3
71	1.7	2.4	1.4	0.9	0.9	2.8
73	3.1	4	3.5	2.1	3.5	2.2
74	1.7	2.5	1.4	1.5	1.7	1.8
74	1.7	2.5	1.4	1.5	1.7	1.8
76	1.4	2.1	1	1.2	0.5	0.8
77	3.3	3.5	1.8	2.3	3	2.5
78	2.3	3	2.5	2.8	3.5	2.8
79	2.3	3.5	3.1	2	3.7	2.8
80	3.2	3.5	2.9	1.8	3.6	2.5
81	1.3	2.1	1.3	0.7	0.8	0.6
82	1.8	3.3	2.2	1.8	1.5	1.8
83	1.3	2.9	0.5	0.6	0.5	0.5

Table A10 (Cont'd)

Lines	G/3w	G/4w	REP1w3	REP2w3	REP1w4	REP2w4
84	3	3.5	2.6	2.1	3.2	3.1
85	1.6	2.4	1.1	1.1	0.5	1.2
86	2.2	3	1.4	1.1	0.8	1
87	2	2.7	1.3	0.8	0.5	0.7
88	3.2	3.5	3	3.3	3.3	3.3
89	1.4	2.6	1	0.7	0.5	0.7
90	2.3	3.5	3	3.4	4	3.1
91	2.5	3.5	3.5	3.4	3.8	2.9
92	1.1	2.2	2.1	0.7	1.3	0.7
93	1.6	2.3	1.3	1.8	0.9	1
94	1.9	2.6	1.4	1.9	0.9	1
95	0.9	1.7	1.2	1.6	0.7	1.6
96	0.8	1.8	1	1.4	0.5	0.6
97	2.7	3.5	3.3	3	3.3	3.2
98	1.2	2.8	1.7	1.5	2.1	1.1
99	2.9	3.4	2.9	3.3	2.4	3.2
100	2.1	3.4	2.5	2.8	2.7	3.3
101	1.1	1.8	0.6	0.8	0.9	0.7
102	3.4	3.6	3.3	2.9	2.5	2.7
103	1.9	3	1.6	2.3	1.1	1.3
104	3.2	3.5	3.1	2.6	3.4	2.7
105	3	3.4	3.4	2.4	3.4	3.1
106	1.1	3.4	1.6	0.9	1.1	0.9
107	2.8	3.3	2.5	1.9	2.6	1
108	1	3.3	2.2	1.5	1.7	1.2
109	1.8	3	1.1	0.6	0.6	0.7
110	2.2	2.7	1.6	1.2	1.1	1.1
111	2.4	3	3	2.4	3.5	3
112	2.1	2.4	3.2	2.3	2.4	2.5
113	0.5	2.1	1.3	0.8	0.8	0.8
114	2.9	3.5	2.9	2.3	3.6	2.5
115	3.3	3.5	2.8	2.5	3.6	2.6
116	0.5	1.3	0.6	0.5	0.5	0.5
117	0.5	1.7	0.8	0.5	0.5	0.5
118	2.3	2.8	3.2	2.8	3.1	3.1
119	1.4	1.5	0.7	1.6	0.5	1
120	1.6	1.8	2	1.5	0.9	1.1
121	1.3	2.7	1.6	1.3	0.6	1.1
122	2.8	3.1	2.5	1.9	3.3	2.6
123	0.8	2	0.9	0.5	0.7	0.8
124	1.9	2.2	1.6	0.7	0.6	0.8

Table A10 (Cont'd)

Lines	G/3w	G/4w	REP1w3	REP2w3	REP1w4	REP2w4
125	1.8	2.3	2	1.7	1.8	1.8
126	1.4	2.3	1.6	1.4	1.4	0.8
128	1.9	3	2.4	2.9	2.3	2.2
129	0.6	1.6	1.3	1	1	0.6
130	1.7	3	2.1	1.5	1.6	1.2
131	1.3	2.5	2.3	3.3	2	3.5
132	3.5	3.5	3.3	3.1	3.4	3.2
133	3.5	3.5	3.5	3.5	3.6	3.5
134	3.3	3.5	3.2	3	3	3.3
135	2.9	3.5	3.2	3.1	2.2	2.9
136	3.5	3.5	3.3	3.5	3.2	3.4
137	1.6	2.5	0.9	0.6	0.6	0.5
138	3.5	3.5	3.4	3.1	3.5	3.3
139	3.2	3.5	2	2.8	2.2	3.1
140	2	2.3	1	1.4	1	1
141	3.4	3.5	3.1	2.9	3.5	2.8
142	1.9	2.3	1.2	0.5	0.8	0.6
143	2.5	3.5	1.4	1	0.9	1
144	1.3	1.8	1.9	0.9	0.8	1.1
145	1	1.7	0.9	0.7	0.6	0.6
146	1.4	1.8	0.7	0.5	0.5	0.5
147	3.5	3.5	3.2	2.5	3	2.8
148	1.1	2.7	1.5	0.8	1.3	0.7
149	0.6	1.4	0.6	0.6	0.5	0.5
150	1.1	1.3	0.7	0.5	0.5	0.5
151	2.1	3	1.4	0.5	0.8	0.5
152	3.4	3.5	3.3	2.7	3.8	2.8
153	2.3	3.4	2	1.9	0.9	1.1
154	1	1.8	1.4	1.4	0.6	0.9
155	3.7	3.5	3.2	3.4	3	3.3
156	3.4	3.7	2.9	2.6	2.2	3.5
157	1.1	2.2	0.5	0.6	0.7	0.8
158	3.4	3.5	3	2.8	3	3.3
159	1.5	2	2.8	1.3	2.5	1.5
160	3.7	3.8	2.6	2.3	3.1	2.3
161	1.6	2	1	0.8	1.8	0.5
162	1.4	1.3	0.6	0.5	0.5	0.5
163	3.4	3.5	2.6	2.9	1.8	3.3
164	1.5	1.5	1.6	1.5	1	1
165	3.4	3.5	3.2	3.1	2.6	2.4
166	3.4	3.5	3.1	2.8	3.7	2.9

Table A10 (Cont'd)

Lines	G/3w	G/4w	REP1w3	REP2w3	REP1w4	REP2w4
167	1	2.2	1	1.4	2	0.5
168	2.5	3.5	3.1	2.1	3	1.8
169	1.7	1.7	0.8	0.6	0.7	0.7
170	1.5	2	1.3	0.6	1	0.6
171	1.7	2.5	1.8	1.1	1.6	1.1
172	1.9	3	2.6	3	3.3	2.7
173	1.9	3	2.3	3.2	2.6	2.7
174	3.5	3.5	3.2	2.3	3.2	2.3
175	3	3	0.5	0.7	1	1.5
176	2	3.3	2.8	1.7	3.2	2.4
177	0.9	1.5	1.6	1	1.3	1.1
178	1.1	1.8	0.8	0.5	0.5	0.5
179	3.2	3.5	2.8	3.2	3.3	3.2
180	2.6	2.6	2.8	3	1.6	1.7
181	0.7	1.4	0.9	1.1	0.5	0.5
182	2.1	3.5	3.3	3	3	2.9
183	2	3.5	3.4	2.8	3.3	2.3
184	3.5	3.5	3.2	2.6	3.5	2.4
185	3.5	3.5	1.8	2.9	0.5	2.3
186	2.4	3	2.8	2.2	3.5	2.4
187	2	3	2.9	2.8	3.1	2.6
188	1.5	2.1	1.4	1.4	0.7	1.1
189	0.7	1.1	0.8	0.7	0.5	0.6

Table A11 Aphid resistance scoring for 070063 population in the field

RIL No.	1	2	3	4	5	6	7	8
1	3.5	3.5	3.5	3.5	3.5			
2	3.5	3.5	3.5	3.5				
3	0.5	1	1.5	1	1.5	1		
4	3.5	1.5						
5	3.5	3.5	3.5	3.5				
6	-							
7	3.5	3.5						
8	3.5	3.5						
9	1	1.5	2	1.5	1.5	1.5		
10	1.5	1.5	3.5	3.5				
11	3	3.5						
12	0.5	1						
13	3	2	1.5					
14	3.5	2.5	3.5	3.5	3.5			
15	3.5	3.5	3.5	3.5				
16	3.5	3.5	3.5	3.5	3	2	1.5	
17	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
18	1.5	0.5	1	0.5	0.5	0.5		
19	3.5	3.5						
20	3							
21	3.5	3.5	3.5					
22	3	3	3					
23	0.5	0.5	0.5					
24	3.5							
25	3.5							
26	2.5	2						
27	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
28	1.5	1	2	1.5	0.5	2		
29	1	0.5	1	0.5	1	1	0.5	
30	3.5	3.5	3.5	3.5	3.5	3.5	3.5	
31	2	2	1	1.5	1	1.5	2	
32	0.5	0.5	0.5	0.5	0.5			
33	2	3.5	3.5	3.5				
34	1.5	1.5	2	1.5	2	1	1.5	1.5
35	3.5	3.5	3.5	3.5	3.5	3.5	3.5	
36	3.5	3.5	3.5					
37	2	2	2					
38	3.5	3.5	3.5	3.5	3.5	3.5		
39	1.5	2	1	1.5	2	1.5	1.5	
40	1	1.5	1	1.5	1	1	1	
41	3.5	3.5	3.5	3.5	3.5	3.5		

Table A11 (Cont'd)

RIL No.	1	2	3	4	5	6	7	8
42	3.5	3.5	3.5	3.5	3.5			
43	3.5	3.5	3.5	3.5	3.5			
44	1	1.5	1.5	1	1.5	1.5		
45	3.5	3.5	3.5	3.5	3.5	3.5	3.5	
46	2	1.5	2	1.5	1.5			
47	1	0.5	1.5	0.5	0.5			
48	0.5	0.5	0.5	0.5	0.5	0.5		
49	0.5	0.5	0.5	0.5	0.5	0.5		
50	0.5	0.5	0.5	0.5	0.5	0.5	1	
51	3.5	3.5	3.5					
52	3.5	3.5	3.5	3.5	3.5	3.5	3.5	
53	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
54	0.5							
55	3.5	3.5	3.5	3.5				
56	0.5	1	1	0.5	1.5	0.5	0.5	1.5
57	3	3	3.5	3.5	3			
58	2	1.5	1.5	2	1.5	1		
59	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
60	0.5	0.5	0.5	0.5	0.5	0.5	1.5	
61	3.5	3.5	3.5	3.5	3.5			
62	2	1.5	1.5	2	1.5	1.5		
63	2	2	1.5	2	2			
64	3.5	3.5	3.5	3.5				
65	3.5	3.5	3.5	3.5	3.5	3.5	3.5	
66	2.5	2	2	2.5	1.5	2	2.5	
67	3.5	3.5	3.5					
68	2.5	2	2.5	2	2.5			
69	3.5	3.5	3.5	3.5	3.5	3.5	3.5	
70	3.5	3.5	3.5					
71	3.5	3.5	3.5					
72	2	3	2	3	2	2	2	
73	3.5	3.5	3.5	3.5	3.5			
74	1.5	1.5	2	2	1	1.5		
75	3	3	2	2	3.5	3.5		
76	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
77	3.5							
78	3.5	3.5	3.5	3.5				
79	0.5	0.5	0.5	0.5				
80	0.5	0.5	0.5					
81	2	3.5						
82	0.5	0.5	0.5	0.5				

Table A11 (Cont'd)

RIL No.	1	2	3	4	5	6	7	8
83	0.5	1						
84	3.5	3.5	3.5					
85	1	1						
86	3.5							
87	3.5	3.5	3.5					
88	3.5	3.5						
89	0.5	0.5	0.5	0.5	0.5	0.5		
90	2	0.5	0.5	0.5				
91	3.5	3.5	3.5					
92	0.5	0.5	0.5					
93	3.5	3.5	3.5					
94	3.5	3.5	1.5	1.5	2			
95	3.5	3.5	3.5	3.5	3.5			
96	3.5	3.5	3.5	3.5				
97	3.5	3.5	3.5	3.5				
98	3.5	3.5	3.5	3.5				
99	2	2.5	2	2.5				
100	2	1.5	2	1.5				
101	3.5	3.5	3.5	3.5	3.5			
102	0.5	0.5						
103	1	0.5	0.5					
104	3.5	3.5	3.5					
105	3.5	3.5	3.5					
106	3.5	3.5	3.5	3.5				
107	0.5	0.5	0.5	0.5	0.5			
108	3.5	3.5						
109	0.5	0.5	0.5					
110	1.5	1.5	1.5	2	2			
111	3.5	3.5	3.5	3.5	3.5			
112	3.5	3.5	3.5	3.5				
113	2	2.5	2	2.5	2.5			
114	2	2						
115	3.5	3.5	3.5	3.5				
116	0.5	0.5	0.5	0.5				
117	0.5	0.5	0.5	0.5				
118	3.5							