# GENETIC ANALYSIS OF SOYBEAN APHID RESISTANCE GENE IN SOYBEAN K1621

by

JIANYE MENG

B.S. Nanjing Agricultural University, 2004

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2010

# Abstract

The soybean aphid (*Aphis glycines* Matsumura) has been one of the major pests of soybean [*Glycine max* (L.) Merr.] in soybean-growing regions of North America since it was first reported in 2000. The objectives of this study were to screen for soybean aphid resistant genotypes, determine the inheritance of resistant genes, and map and validate the resistance gene in the moderate resistant genotype K1621 using simple sequence repeat (SSR) markers. A mapping population of 150 $F_{2:3}$ families from the cross between K1621 and susceptible genotype KS4202 were evaluated for aphid resistance. Phenotyping was conducted on the basis of total aphid number per plant 7 days following infestation with 4 aphids. Inheritance study indicated that one major dominant gene controls soybean aphid resistance in K1621. After SSR markers for polymorphism were screened between parents, a total of 133 polymorphic markers distributed across the soybean genome were used for genotyping. One quantitative trait loci (QTL) controlling antibiotic resistance was found by using the composite interval mapping method. This QTL localized on chromosome 13 (linkage group F) between markers Sat_234 and S6814 and explained 54% of the phenotypic variation. The putative QTL was further validated by single marker analysis using an independent population derived from the cross of K1621 and Dowling. The locus for soybean aphid resistance in K1621 was named [*Rag*]_K1621. The markers identified and validated in this study could be useful for marker-assisted selection of [*Rag*]_K1621.

# GENETIC ANALYSIS OF SOYBEAN APHID RESISTANCE GENE IN SOYBEAN K1621

by

JIANYE MENG

B.S. Nanjing Agricultural University, 2004

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2010

Approved by:

Major Professor
William T. Schapaugh, Jr.

# Copyright

# Abstract

The soybean aphid (*Aphis glycines* Matsumura) has been one of the major pests of soybean [*Glycine max* (L.) Merr.] in soybean-growing regions of North America since it was first reported in 2000. The objectives of this study were to screen for soybean aphid resistant genotypes, determine the inheritance of resistant genes, and map and validate the resistance gene in the moderate resistant genotype K1621 using simple sequence repeat (SSR) markers. A mapping population of 150 $F_{2:3}$ families from the cross between K1621 and susceptible genotype KS4202 were evaluated for aphid resistance. Phenotyping was conducted on the basis of total aphid number per plant 7 days following infestation with 4 aphids. Inheritance study indicated that one major dominant gene controls soybean aphid resistance in K1621. After SSR markers for polymorphism were screened between parents, a total of 133 polymorphic markers distributed across the soybean genome were used for genotyping. One quantitative trait loci (QTL) controlling antibiotic resistance was found by using the composite interval mapping method. This QTL localized on chromosome 13 (linkage group F) between markers Sat_234 and S6814 and explained 54% of the phenotypic variation. The putative QTL was further validated by single marker analysis using an independent population derived from the cross of K1621 and Dowling. The locus for soybean aphid resistance in K1621 was named [*Rag*]_K1621. The markers identified and validated in this study could be useful for marker-assisted selection of [*Rag*]_K1621.

# Table of Contents

# List of Figures

# List of Tables

# Acknowledgements

I am heartily thankful to my advisor, Dr. William T. Schapaugh Jr., who gives me encouragement, supervision and support from the beginning to the concluding level of my studies and research. Without him, it would be impossible for me to finish this work. I would like to express my appreciation to Dr. John Reese, Dr. Michael Smith and Dr. Jianming Yu for serving on my committee and providing helpful guidance and advice.

I would like to thank Dr. Paul St. Amand for giving me technical guidance and answering my questions. My gratitude also goes to Russell Dille, for his professional assistance, handy invention, and excellent tips for my experiments. I am blessed to know and become a lifetime friend with Pam Rzodkiewicz since my first day in the United States. Her support, encouragement and friendship helped me get through a lot of difficulties. I thank my fellow graduate students in soybean breeding program, Junghoon Lee, Levi Walker and Lilly Brzostowski, for their help and discussion for my project. I also thank colleagues from the Department of Entomology, especially Sharon Starkey and Xiang Liu, for their great cooperation during my stay in Dr. Smith's lab. Thanks also go to the undergraduate student workers who helped me with my greenhouse work.

I thank my parents and my sister for their endless love and support. I would not be able to go this far without them. My final appreciation goes to my fiancé Yuan for always standing behind me, giving me support, and bringing me joy and happiness.

# CHAPTER 1 - Literature review

## INTRODUCTION

The Soybean Aphid (*Aphis glycines* Matsumura) (SBA) is a soybean pest introduced to North America in the summer of 2000 (Hartman et al. 2001). From then on, the infested soybean-growing area has spread as far south as Mississippi and Georgia in 2005 and as north as three Canadian provinces (Venette and Ragsdale 2004). In August of 2002, the SBA was first reported in five eastern Kansas counties (Sloderbeck et al. 2003). In September of 2004, the SBA was detected in 64 Kansas counties. Some soybean field in northeastern Kansas was sprayed as the SBA population hit the economic injury threshold. From 2005 to 2007 SBA populations were relatively low with no reports of serious injury. However, in 2008 populations were again well established in northeast Kansas (Whitworth et al. 2010).

The SBA has been the most serious threat to soybean production in China (Wang et al. 1962, 1996; Yue et al. 1989; Wu et al. 1999; Sun et al. 2000). Adults and nymphs of SBA cause plant damage and yield loss by both extracting phloem sap from host plant and transmitting viruses (Guo and Zhang 1989; Wang et al. 1998).

The SBA can be controlled by chemical insecticide, natural enemies, and plant resistance genes. Compared with other methods, host plant resistance is more efficient, economical and environmentally friendly. In addition, plant resistance is an integral component of an integrated pest management (IPM) system (Auclair 1989) and has been shown to be highly compatible with other control methods.

SBA resistance genes can be used in plant breeding by conventional backcrossing and selection or by marker assisted selection. Molecular markers that are tightly linked to the SBA

resistance gene will help select desired individual progeny quickly and accurately. In this study, Kansas soybean lines were screened for SBA resistance (Chapter 2). One of the identified resistant lines, K1621, was then investigated for the character and inheritance of the resistance gene (Chapter 3). One major QTL that controls SBA resistance was identified in K1621 using quantitative trait loci (QTL) mapping and validation (Chapter 4).

# SOYBEAN

## *Economic importance*

The soybean [*Glycine max* (L.) Merr.] is a species of legume cultivated for protein and oil. Native in East Asia, soybean has been grown by farmers in China for 5,000 years. Soybean is widely used to produce meal, feed, oil, and biodiesel fuel. In the United State, soybean production has increased from 1,861 million bushels in 1984 to 3,361 million bushels in 2009. Soybean crop value was $ 31,760 million in 2009, which is almost three times of the crop value in 1984. Today farmers in more than 30 states grow soybeans, making soybean the United States' second largest crop in cash sales and the number one value crop export (SOYSTATS 2010).

## *Soybean genome*

The dense genetic marker map of soybean (Choi et al. 2007; Song et al. 2004) has been used extensively in QTL mapping studies for more than 90 distinct traits (Schmutz et la. 2010). However the functional gene or transcription factor underlying the QTL is unknown in most cases. Most recently, the soybean genome was sequenced by a whole-genome shotgun approach and integrated with physical and high-density genetic maps (Schmutz et la. 2010). The 1.1-gigabase genome sequence is assembled into 20 chromosomes. Among the predicted 46,430

protein-coding genes, 75% of them are duplicated. The available soybean genome sequence will allow cloning and identification of the genes that are already mapped (Meyer et al. 2009). In addition, comparative genomics approach can be used between soybean and other species to facilitate gene identification.

## SOYBEAN APHID (*Aphis glycines* Matsumura)

### *Morphology and biology*

The soybean aphid (*Aphis glycines* Matsumura) (SBA) is a small (adult size is 1/16 in), light yellow or yellowish green aphid with two distinct black cornicles at the tip of its abdomen. SBA is morphologically and biologically similar to other aphids. For a long time SBA was mistaken for the cotton aphid (*Aphis gossypii* Glover). Zhang and Zhong (1982) showed that the offspring of the cross between *A. glycines* and *A. gossypii* could reproduce parthenogenetically and sexually and complete their life cycle. Voegtlin et al. (2004) compared *A. glycines*, with *A. gossypii* and *A. nasturtii* Kaltenbach that have the same overwinter host. In general, most characters of *A. glycines*, including alate spring migrants, apterous viviparous females and alate viviparous females, are not as distinct as those of the other two species. However, a combination of body color, black cornicles, and its colonization on soybean distinguishes *A. glycines* from other aphid species.

The SBA has a typical heteroecious holocyclic life cycle (Zhang and Zhong 1982; Ragsdale et al. 2004). During its life history, SBA alternate hosts between primary host buckthorn (*Rhamnus*) and secondary host cultivated soybean (*G. max*). Wild Glycine species can also serve as secondary host for SBA (Wang et al. 1962). The SBA overwinters as eggs under the buds of buckthorn. In spring nymphs hatch and develop into wingless females (fundatrices).

The following generations on buckthorn consist of wingless (apterous viviparous) females and winged (alate viviparous) females, which then emigrate to cultivated soybean field. During the summer, SBA reproduces parthenogenetically on soybean plants for 15 to 18 overlapping generations. Both wingless and winged females are produced on soybean to build up colony and migrate to other host plants throughout the growing season. In autumn, winged females (gynopara) emigrate from soybean to buckthorn and produce nymphs that develop into wingless females (oviparae). At the same time winged male aphids produced on soybean also emigrate to buckthorn in search of oviparae. Eggs are produced after males and oviparae mate and over winter on buckthorn (Fig. 1.1).

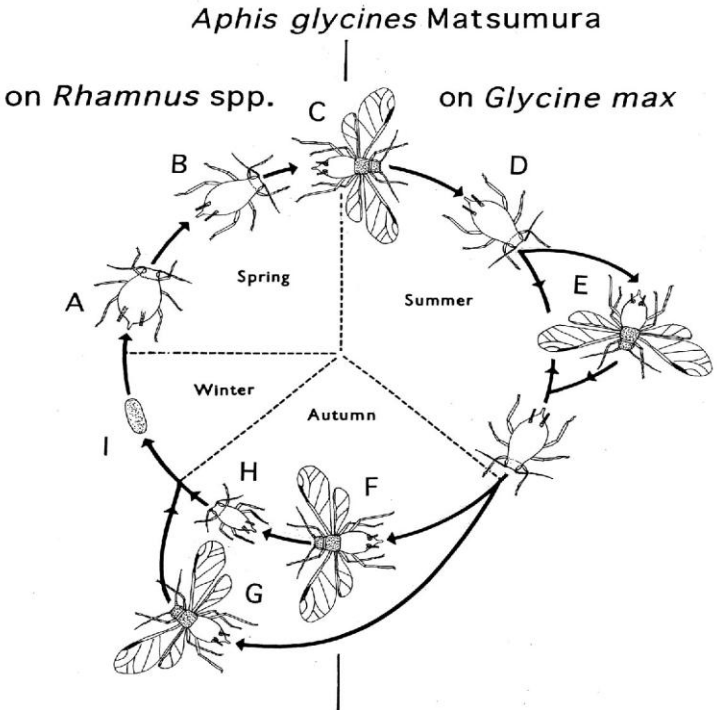



**Figure 1.1 Life cycle of the soybean aphid (Ragsdale et al. 2004).**

(A) Fundatrix on Rhamnus. (B) Apterous viviparous female on Rhamnus. (C) Alate viviparous female, spring migrant from Rhamnus to soybean. (D) Apterous viviparous female on soybean. (E) Alate viviparous female, summer migrant. (F) Gynopara, fall migrant from soybean to Rhamnus. (G) Male migrates from soybean to Rhamnus. (H) Ovipara, on Rhamnus. (I) Overwintering egg on Rhamnus.

### *Origin and distribution*

Soybean aphid was native of Asia and widely distributed in the major soybean-growing regions of China (Wang et al. 1962). The SBA has also been reported as a pest in Korea (Chung et al. 1980), Japan (Takahashi et al. 1993), the Philippines (Quimio and Calilung 1993), Thailand (Paik 1963), Indonesia (Iwaki 1979), Malaysia (Blackman and Eastop 2000), India (Takahashi et al. 1993), Russia (CAB International 2001) and Australia (Fletcher and Desborough 2000).

In the North America, soybean aphid was first found in the Great Lakes area of the United State in the summer of 2000 (Alleman et al. 2002) and in Canada in 2001 (Baute 2002). From 2000 to 2009 the SBA has migrated into all major soybean-growing areas of North America. The first major impact occurred in 2001 and then the pest has broken out of every-other-year pattern. During the 2009 growing season, the SBA developed into economic populations in eastern region (New York and Ontario, Canada) began in July continuing through August, as well as in Midwestern region (Minnesota, Wisconsin, and Iowa) during August. In some affected areas in S. Illinois and Missouri, the SBA populations reached or exceeded economic threshold (Voegtlin and Cullen 2009). The North Central IPM Center built up a regional soybean aphid suction trap network to monitor fall flight of SBA back to its overwintering host, and make prediction of the outbreak in the following year.

### *Damage and economic loss caused by SBA*

Reproduction of the SBA on soybean is affected by temperature. The optimum temperature for reproduction is between 72 to 77°F with the relative humidity below 78 percent. A population can double in size between 2.7 and 13.4 days, depending on the temperature (Ragsdale et al. 2007). Adults and nymphs of the SBA use their piercing/sucking mouthparts to extract phloem sap from stems, leaves and pods of soybean plants. The direct feeding causes leaf

distortion, chlorosis, plant stunting, reduced pod set and decreased seed weights (He et al. 1991; Wang et al. 1962; Wu et al. 1999).

A byproduct of SBA feeding is secreted out of their bodies as honeydew that sticks on stems and the surface of leaves and permits the growth of sooty mold, which turns the leaves dark and interferes with plant photosynthesis (He et al. 1991; OMAFRA 2002), thus leads to yield loss and quality reduction (Lin et al. 1993).

In addition to direct feeding, the SBA is an efficient transmitter of several viruses, including alfalfa mosaic virus, bean yellow mosaic virus peanut stunt virus, tobacco ringspot virus (Clark and Perry 2002), soybean mosaic virus (Clark and Perry 2002; Burrows et al 2005), and potato virus Y (Davis et al. 2005). Soybean mosaic virus is of major concern because it causes yield loss and decrease seed quality (Wu et al. 2004).

Yield loss caused by SBA was over 50% in heavily infested fields in China (Wang et al. 1998). Ostlie (2002) reported almost a 50% yield loss in southeast Minnesota in 2001. Study of Beckendorf et al. (2008) showed that aphid numbers and the soybean yield and components had strong negative linear relationships. In addition to yield loss, SBA feeding affects seed oil and protein concentration (Beckendorf et al. 2008). The maximum possible yield loss is as high as 75% and 48% for SBA infestation starting at V5 stage and R2 stage, respectively (Catangui et al. 2009).

### *Strategies for control of SBA*

The strategies of SBA management include chemical control, biological and cultural control, and host plant resistance. Chemical control is the most used method for controlling SBA in China (Wang et al. 1998; Ye et al. 1996). The use of insecticides has increased in the North Central region of the United States to control SBA since its arrival and establishment

(NASS/USDA 2005). Foliar-applied pyrethroid and organophosphate insecticides are the major chemicals for SBA management in North America (Johnson et al. 2009). Seed treatment with neonicotinoid insecticide is another option to control SBA with the ease of use and protection from early season insect pests (Bradshaw et al. 2008). Currently the widely accepted economic threshold for spraying is 250 aphids per plant between flowering (R1) and early seed set (R5) (Ragsdale et al. 2007). Though chemical control is fast and effective, the increased use of insecticides not only causes environmental problems, but also kills natural enemies and promotes pest resistance.

An alternative method to control SBA is to use natural enemies. A number of natural enemies of SBA have been reported in China, including parasitoids, predators, and pathogen hyperparasitoids (Wu et al. 2004). Some of the species are also present in the United States, including Asian lady beetle (*Harmonia axyridis*), insidious flower bugs (*Orius insidiosus*) (Rutledge et al. 2004), and parasitoids (*Aphidius sp.*) (Nielsen and Hajek 2005). Predators help suppress SBA population growth in June and July when fields have small aphid population. But if the population is higher than 200 aphids per plant, the impact of these predators is limited. A strain of parasitoid wasp *Binodoxys communis* (Gahan) was collected from soybean aphid in China and released in the soybean field in North America in 2007. This species appears to be well adapted and exhibits fairly high levels of host specificity (Wyckhuys et al. 2007).

Cultural control is considered as an important method to manage SBA. The impact of various controllable factors, including cropping system, soybean variety, sowing time, and fertilizer and pesticide application on the soybean aphid has been analyzed by Wang and Ba (1998) and Wang et al (2000). By interplanting soybean and maize(4:1) or sowing soybean and maize seeds (9:2) in the same holes, soybean yield significantly increased and *A.glycines* was

effectively controlled by natural enemies (Wang et al. 2000). In addition, early planting was thought to allow soybeans to escape or delay aphid population buildup.

Compared with chemical and biological control, host plant resistance is an efficient, economical and environmentally friendly method for controlling SBA. In addition, plant resistance is an integral component of an integrated pest management (IPM) system (Auclair 1989) and has been shown to be highly compatible with other control methods (Smith 2005). Research of soybean resistance to SBA in the United States started shortly after the arrival of the pest. Hill et al. (2004a) studied SBA colonization on *Glycine* species and other legumes. The colonization was observed on *Glycine* species, but not on other legumes. After screening over 1500 soybean genotypes, Hill et al. (2004b) found SBA resistance in seven soybean genotypes. The soybean cultivars Jackson, Dowling, and PI 71560 are highly resistant to SBA (Hill et al. 2004b; Li et al. 2004).

To prevent and manage SBA breakout, more than one strategy should be organized into an IPM system to reach the efficiency and reduce side effects.

## SOYBEAN RESISTANCE TO SOYBEAN APHID

### *Categories and mechanisms of plant resistance*

Plant resistance is genetically inherited qualities that make plants suffer lesser damage compared to those without these qualities, when challenged by insects (Painter 1951; Smith 1989). There are three categories of plant resistance to insects: Antixenosis, Antibiosis, and Tolerance. Often there is overlap between the antibiosis and antixenosis. Two or more categories may work together in one resistant plant (Smith 1989).

Antixenosis, also known as nonpreference resistance, leads to a plant acting as a poor host and the pest then selects an alternate host plant (Smith 2005). Antixenosis can be morphological or chemical. Physical barriers, such as thickened epidermal layers, waxy coatings on leaves and stems, or trichoma can force insects to feed on other host plant. Volatile chemicals emitted by the resistant plants, such as alkaloids, terpene lactones, and phenols can act as repellents to insects (Smith 1989). Antixenosis resistance to aphid is usually determined by choice experiment. Free- moving aphids are allowed to feed on plants of different genotypes. The plants with larger colony of aphid are more susceptible than those with fewer aphids.

Antibiosis resistance affects adversely the biology of an insect pest that is attempting to feed on the plant (Smith 2005). Both chemical and morphological plant defenses mediate antibiosis. When some toxins and inhibitors are present or the levels of essential nutrients decrease, antibiosis occurs. Non-choice experiments are often used to determine plant antibiotic resistance. The movement of aphid is restricted in a certain area on the plant. Intrinsic rate of increase (Hawley et al. 2003; Miller et al. 2003), decreased fecundity and longevity, and increased mortality (Li et al. 2004) of the aphid can be assessed and used as an indicator of antibiotic resistance.

Tolerance is the ability possessed by plant that can withstand or recover from damage caused by insect populations equal to those on susceptible cultivars (Smith 2005). Unlike antixenosis or antibiosis, tolerance is plant character and is not part of an insect-plant interaction. However, tolerance often occurs together with antixenosis and antibiosis. Different from antixenosis and antibiosis, the quantitative assessment of tolerance is often accomplished by evaluating the plant characteristics. Tolerance is usually measured by dry weight loss (DWT) and SPAD (Soil Plant Analysis Development) meter readings (Flinn et al. 1994). Other

measurements, such as main tiller height, number of tillers, chlorosis, leaf rolling, number of florets, and head length are also used in experiments (Miller et al. 2003).

### *Sources of soybean resistance*

Currently, there is no commercial variety with SBA resistance available in the United States. However a large amount of soybean germplasm has been screened for SBA resistance and a few resistant genotypes have been identified (Diaz-Montano et al. 2006; Hill et al. 2004; Mensah et al. 2005; Mian et al. 2008a). Study of Hill et al. (2004b) reported seven genotypes with highly resistance to SBA, of which three ancestors of North American genotypes Dowling and Jackson possess antibiosis resistance and PI 71560 possesses antixenosis resistance. Among the early maturing (maturity group III) germplasms, four genotypes were found resistant to SBA (Mensah et al. 2005), of which PI 567541B and PI 567598B have antibiosis resistance; PI 678543C and PI 678697C have antixenosis resistance. Mian et al. (2008a) identified PI 243540 with antibiosis resistance. Diaz-Montano et al. (2006) screened more than 200 commercial soybean lines and Kansas soybean experimental lines and identified Pioneer 95B97 and K1639 (Schapaugh et al. 2010) as highly antibiotic to SBA. Three other soybean genotypes (K1621, K1613, and K1642) showed moderate resistance.

### *Soybean aphid resistance genes and QTLs*

A single dominant gene in Dowling and Jackson was mapped to the same region in linkage group (LG) M (Hill et al. 2006a, b). The resistance gene in Dowling, between SSR markers Satt435 and Satt463, was named *Rag1* (Li et al. 2007). The gene in Jackson remained unnamed because of its unknown relation with *Rag1*. Shortly after *Rag1* was reported, a new biotype of soybean aphid (Ohio biotype) was identified and believed to be virulent to *Rag1* (Kim

et al. 2008). Later, a resistance gene to the Ohio biotype in PI 243540 was identified and named *Rag2*. Gene *Rag2* was mapped to LG F between markers Satt334 and Sct_033 (Mian et al. 2008b). Most recently, Zhang et al. (2010) reported a new gene in LG J for SBA resistance from PI 567543C and named it *Rag3*. Two quantitative trait loci (QTL) associated with SBA resistance in early maturing germplasm PI567541B were mapped to LG M and F (Zhang et al. 2009). More recently, a single dominant gene controlling resistance to both biotypes in PI200538 was mapped to LG F (Hill et al. 2009) in the same region as *Rag2*.

## QTL MAPPING

### *Phenotyping and genotyping*

Quantitative traits are genetically determined characteristics that can be measured as continuous value, such as plant height and crop yield. In contrast to qualitative traits, which are controlled by one of few genes, quantitative traits are controlled by multiple genes. Quantitative traits are very common and important in biological studies. It is common to study quantitative traits by charactering QTL affecting it. QTL mapping is a process that estimates the number of genes and individual gene effects, detects the location of QTL on chromosomes, and refines the genetic architecture for the quantitative trait. QTL mapping is the first step toward identification of the actual genes. It can be applied to map-based gene cloning and plant and animal breeding to perform selection of a desired trait more efficiently. The theory of QTL mapping was first described by Sax (1923), who noted a complex trait, seed size in bean was associated with a simple monogenic trait, seed coat color. Modern QTL mapping is derived from this idea, with the defined sequences of DNA acting as the linked monogenic markers.

A typical QTL mapping study in plants starts with mapping population buildup. Two parental homozygous lines are crossed and generate heterozygous $F_1$ seeds. Individuals of $F_1$ generation can be crossed with one of the parents (backcross design), or intercrossed and keep selfing several times to get recombinant inbred lines (RIL). RIL have been commonly used in QTL studies because there are little within-line genetic variance. The population size needs to be considered. It is more likely to detect QTL with small effect in larger population (Zeng 1994; Vales et al. 2005).

Two sets of data, phenotyping data and genotyping data, will be collected from the mapping population. The process of measuring traits of interest is called phenotyping. Easy and efficient methods need to be used for phenotyping because the operation could be very costly and time- and labor-consuming. Genotyping is the process of determining the genotype of individual using molecular markers. Commonly used molecular markers include restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) based markers, such as sequence tagged sites (STS), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequenced repeats (SSR or microsatellites), and single nucleotide polymorphism (SNP). Currently SSR and SNP markers are commonly used in QTL mapping studies.

SSR is a small segment of DNA, usually 2 to 5 bp in length that repeats itself a number of times. Some of the major core motifs of soybean SSR markers include ATT, AT, CTT, and CT (Choi et al. 2007; Song et al. 2004). Most SSRs are single-locus markers, and many SSR loci are multi-allelic. These characteristics make SSRs ideal for both creating genetic maps and defining linkage group homology across mapping populations (Song et al. 2004).

### *Genetic map construction*

The first soybean genetic linkage map of molecular markers was reported by Keim et al. (1990) which contained a total of 150 RFLP loci. Two more genetic maps were developed based on RFLP, isozyme, and morphological markers (Lark et al. 1993; Shoemaker and Specht 1995). Cregan et al. (1999) reported a genetic map based on one or more of three populations, containing 606 SR loci, 689 RFLP, 79 RAPD, 11 AFLP, 10 isozyme, and 26 classical loci. For the first time, 20 consensus linkage groups were established, correlating the 20 soybean chromosomes. Song et al. (2004) reported an integrated genetic linkage map of soybean, which contains 1,015 SSRs, 709 RFLPs, 73 RAPDs, 24 classical traits, six AFLPs, ten isozymes, and 12 others. A total of 5551 SNPs were discovered and mapped to soybean genome. These SNP markers saturated the gaps between some SSR markers and provide important resource for QTL discovery and map-based cloning (Choi et al. 2007).

Because genetic markers are frequently polymorphic in one population, but monomorphic in another, researchers construct genetic linkage map of their own based on specific populations. Popular softwares used to construct genetic linkage maps are MapMaker (Lander et al. 1987) and JoinMap (Stam 1993; Van Ooijen and Voorrips 2001).

### *QTL mapping methods*

Based on the maximum-likelihood method, four approaches are commonly used for QTL mapping: single marker analysis (SMA), interval mapping (IM), composite interval mapping (CIM), and multiple interval mapping (MIM).

SMA method is based on ANOVA, or simple linear regression, and performs statistical analysis between single marker and trait value. The calculations are based on phenotypic means and variances with each of the genotypic classes. The SMA was thought less powerful than the

interval mapping methodologies based on two markers and their estimated genetic map because it confounds the QTL effect from the QTL location (Doerge et al. 1997). However, Rebai et al. (1995) found that IM were more powerful than SMA only in certain cases. This finding was confirmed by Coffman et al. (2003) and they concluded that additional genotypic information in the second marker increased the power of two marker approaches. But under certain situations, SMA is equivalent or more powerful than two marker analysis with the advantage of simplicity.

An IM method is used to estimate the position of a QTL within two markers. The calculations are based on maximum likelihood or simple regression (Haley and Knott 1992). In either calculation, a likelihood ratio (LR) is calculated as a test statistic. The LR can be transferred to a LOD score (logarithm (base 10) of odds) at a certain position (Lander and Botstein 1989). Once LOD score exceeded the threshold, there is evidence for a QTL at that location. Some QTL mapping programs can calculate both LR and LOD directly.

The method of CIM is an extension of IM that evaluates the possibility of a QTL in one interval while simultaneously fitting partial regression coefficients for markers elsewhere in the genome that account for variance caused by non-target QTL (Jansen and Stam 1994; Zeng 1994). CIM can remove the bias caused by QTL that are linked to the position being tested. The method gives more power and precision than simple IM. In CIM, cofactors are used to reduce residual variation by controlling for the genetic background. Cofactors are determined by model selection procedures such as forward selection and backward elimination.

MIM uses multiple marker intervals simultaneously to fit multiple putative QTL directly in the model for QTL mapping (Kao et al. 1999). MIM is a method with improved precision and power for mapping QTL. Numbers, locations, effects and epistasis between QTL can be readily estimated and analyzed.

# References


Alleman RJ, Grau CR, Hogg DB. 2002. Soybean aphid host range and virus transmission efficiency. Proc. Wisc. Fertilizer Agline Pest Manage. Conf. (http://www.soils.wisc.edu/extension/FAPM/fertaglime02.htm).

Auclair JL. 1989. Host plant resistance. p. 225–265. *In* A.K. Minks and P. Harrewijn (ed.) Aphids: Their biology, natural enemies, and control. Vol. C. Elsevier, New York.

Baute T. 2002. Soybean aphids. Ontario Ministry of Agriculture and Food (OMAF). http://www.gov.on.ca/OMAFRA/english/crops/facts/soybean_aphid/soybean_aphid.htm

Beckdendorf EA, Catangui MA, Riedell WE. 2008. Soybean aphid feeding injury and soybean yield, yield components, and seed composition. Agron. J. 100: 237-246.

Blackman RL, Eastop VF. 2000. Aphids on the world's crops: an identification and information guide, 2nd ed. Wiley, New York.

Bradshaw JD, Rice ME, Hill JH. 2008. Evaluation of management strategies for bean leaf beetles (Coleoptera: Chrysomelidae) and bean pod mottle virus (Comoviridae) in soybean. J. Econ. Entomol. 101: 1211-1227.

Burrows MEL, Boerboom CM, Gaska JM, Grau CR. 2005. The relationship between Aphis glycines and Soybean mosaic virus incidence in different pest management systems. Plant Dis. 89: 926-934.

CAB International. 2001. Crop protection compendium. http://www.aphis.usda.gov/npb/soybean/aphisglycines.pdf

Catangui MA, Beckdendorf EA, Riedell WE. 2009. Soybean aphid population dynamics, soybean yield loss, and development of stage-specific economic injury levels. Agron. J 101: 1080-1092.

Choi IY, Hyten DL, Matukumalli LK, Song Q, Chaky JM, Quigley CV, Chase K, Lark FG, Reiter RS, Yoon M, Hwang E, Yi S, Yong ND, Shoemaker RC, Tassell CP, Specht JE, Cregan PB. 2007. A soybean transcript map: gene distribution, haplotype and singlenucleotide polymorphism analysis. Genetics 176, 685–696.

Chung KH, Kwon SH, Lee YI. 1980. Studies on the density of soybean aphids in different cultivars, planting date and spacings. Korean J. Crop Sci. 25: 35-40.

Clark AJ, Perry KL. 2002. Transmissibility of field isolates viruses by Aphis glycines. Plant Dis. 86: 1219-1222.

Coffman CJ, Doerge RW, Wayne ML, McIntyre LM. 2003. Intersection tests for single marker QTL analysis can be more powerful than two marker QTL analysis. BMC Genet. 4:10. doi: 10.1186/1471-2156-4-10.

Cregan PB, Jarvik T, Bush AL, Shoemaker RC, Lark KG, Kahler AL, Kaya N, VanToai TT, Lohnes DG, Chung J, Specht JE. 1999. An integrated genetic linkage map of the soybean. Crop Sci 39:1464–1490

Davis, JA, Radcliffe EB, Ragsdale DW. 2005. Soybean aphid, Aphis glycines Matsumura, a new vector of Potato virus Y in potato. Am. J. Potato Res. 81:101-105.

Diaz-Montano J, Reese JC, Schapaugh WT, Campbell LR. 2006. Characterization of antibiosis and antixenosis to the soybean aphid (Hemiptera: Aphididae) in several soybean genotypes. J. Econ. Entomol. 99(5): 1884-1889.

Doerge RW, Zeng Z-B, Weir BS. 1997. Statistical issues in the search for genes affecting quantitative traits in experimental populations. *Statistical Science.* 13:195–219.

Guo, J. Q., and M. H. Zhang. 1989. Study on the important vectors of soybean mosaic virus and their transmission efficiency. Soybean Sci. 8: 55-63

Fletcher MJ, Desborough P. 2000. The soybean aphid, *Aphis glycines*, present in Australia (http://www. agric.nsw.gov.au/Hort/ascu/insects/aglycin.htm).

Flinn M, Smith CM, Reese JC, Gill B. 1994. Categories of resistance to Greenburg (Homoptera: Aphididae) biotype I in *Aegilops tauschii* germplasm. J. Econ. Entomol. 94(2): 558-563.

Haley CS, Knott SA. 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315-324

Hartman GL, Domier LL, Wax LM, Helm CG, Onstad DW, Shaw JT, Solter LF, Voegtlin DJ, D'Arcy CJ, Gray ME, Steffey KL, Isard SA, Orwick PL. 2001. Occurrence and distribution of *Aphis glycines* on soybeans in Illinois in 2000 and its potential control. Plant Health Progress doi:10.1094/PHP-2001-0205-01-HN.

Hawley CJ, Peairs FB, Randolph TL. 2003. Categories of resistance at different growth stages in Halt, a winter wheat resistant to the Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 96: 214-219

He FG, Yan FY, Xin WM, Li XP, Wang YQ, Zhang GX. 1991. Optimal spraying time and economic threshold of the soybean aphid. Acta Phyt. Sinica 18: 155-159.

Hill CB, Kim KS, Crull L, Diers BW, Hartman GL. 2009. Inheritance of resistance to the soybean aphid in soybean PI 200538. Crop Sci 49:1193-1200

Hill CB, Li Y, Hartman GL. 2004a. Resistance of Glycine species and various cultivated legumes to the soybean aphid (Homoptera: Aphididae). J. Econ. Entomol. 97: 1071-1077.

Hill CB, Li Y, Hartman GL. 2004b. Resistance to the soybean aphid in soybean germplasm. Crop Sci. 44:98-106.

Hill CB, Li Y, Hartman GL. 2006a. A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. Crop Sci. 46:1601-1605.

Hill CB, Li Y, Hartman GL. 2006b. Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. Crop Sci. 46:1606-1608.

Iwaki M. 1979. Virus and mycoplasma diseases of leguminous crops in Indonesia. Rev. Plant Prot. Res. 12: 88-97.

Jansen, RC, Stam P. 1994. High resolution of quantitative traits into multiple loci via interval mapping. *Genetics* 136:1447-1455

Johnson KD, O'Neal ME, Ragsdale DW, Difonzo CD, Swinton SM, Dixon PM, Potter BD, Hodgson EW, Costamagna AC. 2009. Probability of cost-effective management of soybean aphid (Hemiptera: Aphididae) in North America. J. Econ. Entomol. 102: 2101-2108.

Kao CH, Zeng ZB, Teasdale RD. 1999. Multiple interval mapping for quantitative trait loci. *Genetics* 152:1203-1216

Keim P, Diers BW, Olson TC, Shoemaker RC. 1990. RFLP mapping in soybean: association between marker loci and variation in quantitative traits. Genetics 126:735–742

Kim KS, Hill CB, Hartman GL, Mian MAR, Diers BW. 2008. Discovery of soybean aphid biotypes. Crop Sci. 48:923–928.

Lander ES, Botstein D.1987. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

Lark KG, Weisemann JM, Matthews BF, Palmer R, Chase K, Macalma T. 1993. A genetic map of soybean (Glycine max L.) using an intraspecific cross of two cultivars: 'Minsoy' and 'Noir 1'. Theor Appl Genet 86:901–906

Li Y, Hill CB, Hartman GL. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera: Aphididae). J. Econ. Entomol. 97: 1106-1111.

Li Y, Hill CB, Carlson SR, Diers BW, Hartman GL. 2007. Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group M. Mol. Breeding 19:25-34.

Lin CL, Li LT, Wang YP, Xun ZS, Zhang GX, Li SZ. 1993. Effects of the soybean aphid density on major soybean economic characters. Soybean Sci. 12: 252-254.

Mensah C, DiFonzo C, Nelson RL, Wang D. 2005. Resistance to soybean aphid in early maturing soybean germplasm. Crop Sci. 45:2228-2233.

Meyer JDF et al. 2009. Identification and analyses of candidate genes for Rpp4-mediated resistance to Asian soybean rust in soybean (Glycine max (L.) Merr.). Plant Physiol. 150: 295–307.

Mian MAR, Hammond RB, St Martin SK. 2008a. New plant introductions with resistance to the soybean aphid. Crop Sci 48:1055–1061.

Mian MAR, Kang ST, Beil SE, Hammond RB. 2008b. Genetic linkage mapping of the soybean aphid resistance gene in PI 243540. Theor Appl Genet 117:955–962.

Miller HR, Randolph TL, Peairs FB. 2003. Categories of resistance at four growth stages in three wheats resistant to the Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 96(3): 673-679.

[NASS/USDA] (National Agricultural Statistics Service/U.S. Department of Agriculture 2005. Agricultural chemical usage 2005 Teld crops summary. (http://jan-tng.mannlib.cornell.edu/reports/nassr/other/pcu-bb/agcs0506.txt10 April 2010).

Nielsen C, Hajek AE. 2005. Control of invasive soybean aphid, Aphis glycines (Hemiptera: Aphididae), populations by existing natural enemies in New York State, with emphasis on entomopathogenic fungi. Environ. Entomol. 34: 1036-1047.

(OMAFRA) Ontario Ministry of Agriculture, Food and Rural Affairs. 2002. Soybeans: Soybean aphid. Agronomy Guide for Field Crops (Chapter 4), pub 811. (http://www.omafra.gov.on.ca/english/crops/pub811/4aphid.htm).

Ostlie K. 2002. Managing soybean aphid. University of Minnesota, St. Paul, MN. (http://www.soybeans.umn.edu/crop/insects/aphid/aphid_publication_managingsba.htm).

Paik WH. 1963. Aphids of Korea. Publishing Center of Seoul National University, Seoul.

Painter RH. 1951. Insect resistance in crop plants. MacMillan Co., New York.

Quimio GM, Calilung VJ. 1993. Survey of flying viruliferous aphid species and population build-up of *Aphis glycines* Matsumura in soybean fields. Philipp. Entomol. 9: 52-100.

Rebaï A, Goffinet B, Mangin B. 1995. Comparing powers of different methods for QTL detection. *Biometrics.* 51:87–99.

Ragsdale DW, Voegtlin DJ, O'Neil RJ. 2004. Soybean aphid biology in North America. Ann. Entomol. Soc. Am. 97: 204-208.

Ragsdale DW, McCornack BP, Venette RC, Potter BD, Mac-Rae IV, Hodgson EW, O'Neal ME, Johnson KD, O'Neil RJ, DiFonzo CD, Hunt TE, Glogoza PA, Cullen EM. 2007. Economic threshold for soybean aphid (Hemiptera: Aphididae). J. Econ. Entomol. 100:1258–1267.

Rutledge CE, O'Neil RJ, Fox TB, Landis DA. 2004. Soybean aphid predators and their use in integrated pest management. Ann. Entomol. Soc. Am. 97: 240-248.

Sax K. 1923. The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris.*. *Genetics.* 8:552-560

Schapaugh WT, Todd T, Reese J, Diaz-Montano J, Meng J, and Smith CM. 2010. Registration of K1639-2 soybean germplasm resistant to soybean cyst nematode and soybean aphid. J. Plant Registrations 4: 67-69.

Schmutz J, Cannnon SB, Schlueter J et al. 2010. Genome sequence of the palaeopolyploid soybean. Nature 463: 178-183.

Shoemaker RC, Specht JE. 1995. Integration of the soybean molecular and classical genetic linkage groups. Crop Sci 35:436–446

Stam P. 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Sun B, Liang SB, Zhao WX. 2000. Outbreak of the soybean aphid in Suihua prefecture in 1998 and its control methods. Soybean Bull. 1: 5.

Sloderbeck PE, Reese JC, Whitworth RJ, Smith CM, Higgins RA, Schapaugh WT, Wolf RE, Jardine DJ. 2003. The soybean aphid. MF-2582, Kansas State University Agricultural Experiment Station and Cooperative Extension Service, Manhattan, KS. (http://www.oznet.ksu.edu/library/entml2/MF2582.pdf)

Smith CM. 1989. Plant resistance to insects: A fundamental approach. John Wiley & Sons, New York.

Smith CM. 2005. Plant resistance to arthropods: molecular and conventional approaches. Springer, The Netherlands.

Song QJ, Marek LF, Shoemaker RC, Lark KG, Concibido VC, Delannay X, Specht JE, Cregan PB. 2004. A new integrated genetic linkage map of the soybean. Theor. Appl. Genet. 109:122–128.

SOYSTATS 2010. A reference guide to important soybean facts & figures. 2010. American soybean association. Saint Louis, Missouri. http://www.soystats.com/2010/Default-frames.htm.

Takahashi S, Inaizumi M, Kawakami K. 1993. Life cycle of the soybean aphid *Aphis glycines* Matsusmura in Japan. Jap. J. Appl. Entomol. Zool. 37: 207-212.

Vales, MI, Schon CC, Capettini F, Chen XM, Corey AE, Mather DE, Mundt CC, Richardson KL, Sandoval-Islas JS, Utz HF, Hayes PM 2005. Effect of population size on the

estimation of QTL: a test using resistance to barley stripe rust. *Theor Appl Genet* 111: 1260-1270.

Van Ooijen JW, Voorrips RE. 2001. JoinMap 3.0 software for the calculation of genetic linkage maps. Plant Research International, Wageningen, The Netherlands.

Venette RC, Ragsdale DW. 2004. Assessing the invasion by soybean aphid (Homoptera: Aphididae): Where will it end? Ann. Entomol. Soc. Am. 97: 219-226.

Voegtlin DJ, Halbert SE, Qiao G. 2004. A guide to separating *Aphis glycines* Matsumura and morphologically similar species that share its hosts. Ann. Entomol. Soc. Am. 97: 227-232.

Voegtlin D, Cullen E. 2009. United States soybean aphid commentary - 2009 soybean aphid summary. http://sba.ipmpipe.org/cgi-bin/sbr/public.cgi

Wang YZ, Ba F. 1998. Study on optimum control of the soybean aphid. Acta Phyt. Sinica 25: 152-155.

Wang S, Bao X, Sun Y, Chen R, Zhai B. 1996. Study on the effect of population dynamics of soybean aphid (*Aphis glycines*) on both growth and yield of soybean. Soybean Sci. (China) 15: 243-247.

Wang CR, Chen JG, Guo YR, Gong XY, Xu ZF, Lin C. 1998. Development and control of soybean apid, Aphis glycines, in Heilongjiang province. Soybean Bull. 6:15.

Wang CL, Siang NJ, Chang GS, Chu HF. 1962. Studies on the soybean aphid, *Aphis glycines* Mastumura. Acta Entomol. Sinica. 11:31-44

Wang YZ, Ma L, Wang JZ, Ren XZ, Zhu WL. 2000. Systematic optimum control of diseases and insect pests in summer soybean. J. Ecol. 20: 502-509.

Whitworth RJ, Michaud JP, and Sloderbeck PE. Soybean insect management 2010. MF-743. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, February 2010.

Wu XB, Ni WJ, Liu PJ. 1999. Occurrence and control of the soybean aphid, *Aphis glycines* Matsumura. Chin. J. Niol Control 6:20.

Wu Z, Schenk-Hamlin D, Zhan W, Ragsdale DW, Heimpel GE. 2004. The soybean aphid in China: a historical review. Ann. Entomol. Soc. Am. 97(2): 209-218.

Wyckhuys KAG, Hopper KR, Wu KM, Straub C, Gratton C, Heimpel GE. Predicting potential ecological impact of soybean aphid biological control introductions. *CABI Biocontrol News and Information.* 2007. 28:30–34.

Ye Z, Liu Z, Jin D. 1996. Background information of cooperative IPM project: soybean and soybean insect pests in China. Chinese Academy of Agricultural Sciences CAAS, Beijing, China). (http://www.ksu.edu/issa/aphids/reporthtml/trans20.htm).

Yue DR, Guo SG, Shan YL. 1989. Resistance of wild soybean *Glycine soja* to *Aphis glycines*. I. Screening for resistant varieties. Jilin Agric. Sci. 3: 15-19.

Zeng, ZB 1994. Precision mapping of quantitative trait loci. *Genetics* 136:1457-1468.

Zhang GX, Zhong TS. 1982. Experimental study on some aphid life-cycle patterns. Sinozoologica 2: 7-17.

Zhang G, Gu C, Wang DC. 2009. Molecular mapping of soybean aphid resistance genes in PI567541B. Theor Appl Genet 118:473–482.

Zhang G, Gu C, and Wang D. 2010. A novel locus for soybean aphid resistance. Theor appl genet. 120: 1183-1191.

# CHAPTER 2 - Identification of SBA resistance genotypes from Kansas soybean germplasm

## INTRODUCTION

Soybean aphid (*Aphis glycines* Matsumura) (SBA) has become an invasive pest insect to soybean [*Glycine max* (L.) Merr.] since its arrival in North America in 2000 (Hartman et al., 2001). The SBA causes damage by both direct feeding and transmitting viral diseases. Soybean plants can be infested by SBA at any growing stage and the worst damage occurs when plants are infested in the early seed-setting stage (Catangui et al., 2009). The SBA was first reported in five eastern Kansas counties (Sloderbeck et al. 2003) in 2002. In September of 2004, the SBA was detected in 64 Kansas counties. Some soybean field in northeastern Kansas was sprayed as the SBA population hit the economic injury threshold. From 2005 to 2007 SBA populations were relatively low with no reported crop yield losses. However, in 2008 populations were again well established in northeast Kansas (Whitworth et al. 2010).

Compared with chemical and biological control, host plant resistance is an efficient, economical and environmentally friendly method for controlling SBA. There are three categories of plant resistance to insect: antixenosis, antibiosis, and tolerance (Smith, 1989). Antibiosis is the most desired category in breeding programs because the biology of the insect is strongly negatively affected. To measure antibiotic resistance, non-choice experiments are commonly used, where insects' movement is restricted to a single host. The insect reproduction ability is a good index to show the level of plant antibiotic resistance.

The objective of this study was to screen Kansas soybean lines for SBA antibiotic resistance.

# MATERIALS AND METHODS

## *Soybean aphid colony*

The SBA used in all experiments were originally collected from a Kansas soybean field in Geary County, KS, in the summer of 2002. The SBA colony was maintained on soybean KS4202 plants in a growth chamber under a photoperiod of 14: 10(L: D) h and temperature of 24°C (day) and 20°C (night).

## *Screen test for SBA resistance*

Kansas soybean lines were screened for SBA resistance using non-choice tests. Testing lines, the susceptible check KS4202, and the resistance check Jackson or Dowling were planted individually in 3.8-cm-diam. by 21.0-cm-deep plastic Cone-tainers (Ray Leach Cone-tainer, Hummert International, Earth City, MO) and arranged in a completely randomized design with 3 to 5 replicates in a greenhouse under a photoperiod of 14: 10(L: D) h and temperature of 20-26°C. Plastic trays were placed underneath the plants and filled with water to ensure each plant was watered evenly and consistently. When plants grew to V1 stage (Fehr and Caviness, 1977), two wingless aphid adults were transferred to the upper side of each unifoliolate leaf of each plant. Movement of aphids was restricted in double-sided sticky cages (Converters, Inc., Huntingdon Valley, PA) with an inner oval area of 1.2 $cm^2$. Cages were covered with a piece of mesh cloth right after aphids were put into the cages. The total number of aphids produced on each plant was counted 7d after infestation. Results were separated using t-test with $\alpha$=0.05. Genotypes were considered resistant, when the total aphid number is not significantly different from that on Jackson; and moderate resistant, when the total aphid number is higher than that on Jackson but significantly lower than susceptible check KS4202.

# RESULTS

## *Experiment 1: screening test for SBA resistance (48 lines)*

A total of 48 Kansas soybean lines were screening for SBA resistance in the year of 2005-2006. Numbers of adult and nymph on each plant were counted and total aphid number was calculated as the sum of adults and nymphs. The total aphid number on each genotype ranged from 0 to 100. Susceptible check KS4202 and resistant check Jackson had total aphid number of 66.3 and 0, respectively. None of the testing genotypes was as resistant as Jackson and some genotypes showed moderate resistance (Table 2-1).

**Table 2-1 Experiment 1: screening test for SBA resistance (48 lines)**

| Genotype | total SBA number / plant | | number of adults / plant | | number of nymphs / plant | |
|---|---|---|---|---|---|---|
| K03- 4691 | 100.0 | a† | 14.5 | a-i | 85.5 | a |
| K1630RR | 75.7 | ab | 17.3 | a-d | 58.3 | abc |
| K03- 2037 | 72.5 | abc | 11.0 | a-j | 61.5 | abc |
| K03- 2706 | 72.3 | abc | 7.3 | a-j | 65.0 | ab |
| K03- 2814 | 72.0 | abc | 15.0 | a-i | 57.0 | abc |
| K1623RR | 71.0 | abc | 11.3 | a-j | 59.7 | abc |
| KS4202 | 66.3 | a-d | 10.0 | a-j | 56.3 | a-d |
| K03- 2820 | 66.3 | a-d | 14.7 | a-i | 51.7 | a-e |
| K03- 2801 | 66.0 | a-d | 19.3 | a | 46.7 | a-f |
| K03- 4698 | 64.0 | a-e | 16.0 | a-f | 48.0 | a-e |
| K03- 4689 | 62.0 | a-e | 8.0 | a-j | 54.0 | a-e |
| K03- 4244 | 60.3 | a-e | 14.7 | a-i | 45.7 | a-f |
| K03- 2399 | 59.7 | a-e | 15.7 | a-g | 44.0 | a-f |
| K03- 4686 | 59.3 | a-e | 9.7 | a-j | 49.7 | a-e |
| K1619 | 57.7 | a-e | 13.7 | a-i | 44.0 | a-f |
| K03- 3825 | 56.3 | a-e | 5.7 | b-j | 50.7 | a-e |
| K03- 3496 | 56.0 | a-f | 13.3 | a-i | 42.7 | a-f |
| K03- 4702 | 55.0 | a-f | 11.0 | a-j | 44.0 | a-f |
| K01- 2531 | 54.7 | a-f | 16.3 | a-e | 38.3 | b-f |
| K1631RR | 54.3 | a-f | 18.0 | ab | 36.3 | b-f |
| K03- 4685 | 52.3 | a-f | 9.0 | a-j | 43.3 | a-f |
| K03- 4142 | 51.7 | a-f | 11.7 | a-j | 40.0 | a-f |
| K03- 2839 | 49.3 | a-f | 6.3 | a-j | 43.0 | a-f |
| K1643NRR | 49.0 | a-f | 14.0 | a-i | 35.0 | b-f |
| K03- 2054 | 48.7 | a-f | 8.3 | a-j | 40.3 | a-f |

**Table 2-2 Experiment 1: screening test for SBA resistance (48 lines) (continued)**

| Genotype | total SBA number / plant | | number of adults / plant | | number of nymphs / plant | |
|---|---|---|---|---|---|---|
| K03- 4684 | 48.3 | a-f | 15.3 | a-h | 33.0 | b-f |
| K03- 2688 | 47.7 | a-f | 9.0 | a-j | 38.7 | b-f |
| K03- 2694 | 46.0 | a-f | 17.5 | abc | 28.5 | b-f |
| K03- 4150 | 42.3 | b-f | 10.0 | a-j | 32.3 | b-f |
| K03- 4156 | 38.3 | b-f | 11.7 | a-j | 26.7 | b-f |
| K03- 2788 | 36.0 | b-f | 3.7 | e-j | 32.3 | b-f |
| K03- 3997 | 35.0 | b-f | 5.0 | b-j | 30.0 | b-f |
| K03- 4146 | 32.3 | b-f | 6.7 | a-j | 25.7 | b-f |
| K03- 4331 | 32.0 | b-f | 5.0 | b-j | 27.0 | b-f |
| K03- 2791 | 30.7 | b-f | 3.3 | e-j | 27.3 | b-f |
| K03- 4683 | 30.3 | b-f | 6.3 | a-j | 24.0 | b-f |
| K5502NRR | 29.3 | b-f | 4.0 | d-j | 25.3 | b-f |
| K03- 2832 | 26.0 | b-f | 4.3 | c-j | 21.7 | b-f |
| K03- 2811 | 22.0 | b-f | 5.7 | c-j | 16.3 | c-f |
| K03- 4157 | 21.7 | b-f | 1.7 | ij | 20.0 | b-f |
| K4602NRR | 17.7 | c-f | 2.0 | hij | 15.7 | c-f |
| K03- 4043 | 12.0 | def | 2.0 | hij | 10.0 | def |
| K03- 3821 | 12.0 | def | 3.0 | e-j | 9.0 | ef |
| K1620 | 11.3 | def | 1.7 | ij | 9.7 | def |
| K03- 2897 | 11.0 | def | 2.7 | f-j | 8.3 | ef |
| K03- 3582 | 9.7 | ef | 2.3 | g-j | 7.3 | ef |
| JACKSON | 0.0 | f | 0.0 | j | 0.0 | f |

† Mean aphid numbers followed by different letters are significantly different (P<0.05; t-test).

### *Experiment 2: screening test for SBA resistance (10 lines)*

A total of 10 Kansas soybean lines were screening for SBA resistance in 2006. Numbers of adult and nymph on each plant were counted and total aphid number was calculated as the sum of adults and nymphs. The total aphid number on each genotype ranged from 3.3 to 55.2. Susceptible check KS4202 and resistant check Jackson had total aphid number of 41.7 and 3.3, respectively. None of the testing genotypes was as resistant as Jackson. Six genotypes, K1642, K1613, K1621, K5502NRR, K4602NRR, and K1620 showed moderate resistance (Table 2-2).

**Table 2-3 Experiment 2: screening test for SBA resistance (10 lines)**

| Genotype | total SBA number/ plant | | Number of adults / plant | | Number of nymphs / plant | |
|---|---|---|---|---|---|---|
| JACKSON | 3.3 | a† | 1.7 | a | 1.7 | a |
| K1642 | 19.4 | b | 3.2 | ab | 16.2 | b |
| K1613 | 19.0 | b | 4.2 | abc | 14.8 | b |
| K1621 | 23.0 | bc | 4.2 | abc | 18.8 | bc |
| K5502NRR | 21.8 | bc | 4.8 | bcd | 17.0 | bc |
| K4602NRR | 29.7 | bcd | 5.7 | bcd | 24.0 | bcd |
| K1620 | 35.4 | cd | 6.6 | cde | 28.8 | cd |
| K03-4150 | 43.0 | de | 9.5 | ef | 33.5 | de |
| KS4202 | 41.7 | de | 7.3 | def | 34.3 | de |
| K03-2706 | 55.2 | e | 10.2 | f | 45.0 | e |

† Mean aphid numbers followed by different letters are significantly different (P<0.05; t-test).

### *Experiment 3: segregation of K1639*

Genotype K1639 showed similar resistance to SBA as Jackson in previous study (Diaz-Montano 2006). However, heterogeneity was observed in the population. In 2005 eight $F_{8:9}$ progeny of K1639 were evaluated for SBA resistance. Segregation for aphid resistance among eight lines was observed. Total aphid number ranged from 4.2 on K1639-3 to 35.0 on K1639-1. Susceptible check KS4202 had total aphid number of 46 and the number is not significantly different from that on K1639-1. Line K1639-1 was used as susceptible resource in future study. K1639-2 was released as germplasm resistant to SBA and soybean cyst nematode (Chapter 5).

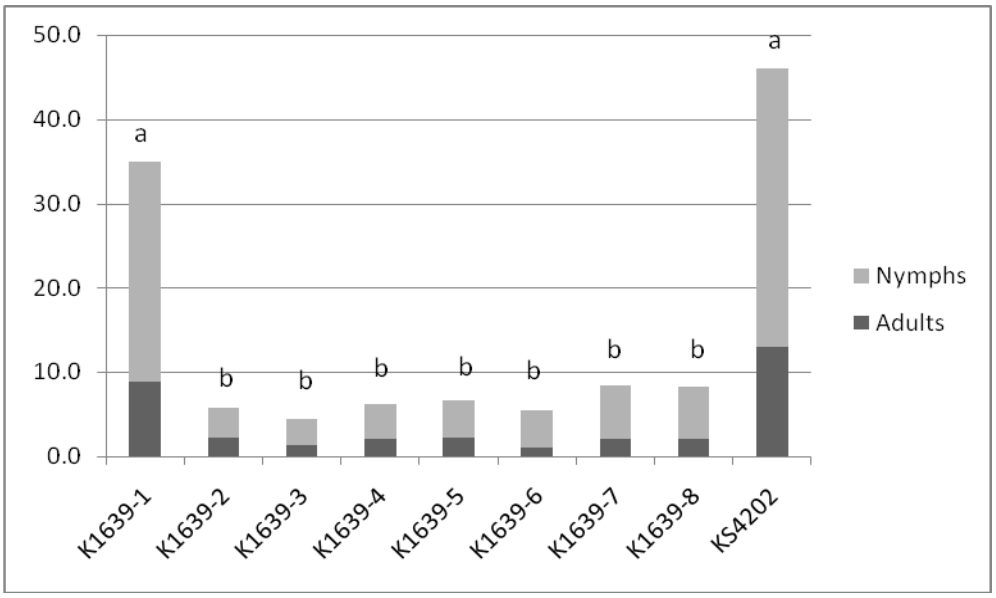



**Figure 2.1 Segregation of 8 progeny lines of K1639. Letters indicate significant difference of total aphid number ($p<0.05$; t-test).**

### *Experiment 4: screening test for SBA resistance (15 lines)*

A total of 15 Kansas soybean lines were screening for SBA resistance in 2009. The total aphid number on each genotype ranged from 0 to 47.6. Susceptible check KS4202 and resistant check Jackson had total aphid number of 19 and 0, respectively. Eleven genotypes, K07-4065, K07-4018, K07-4063, K07-4030, K07-4076, K07-4016, K07-4067, K07-4100, K07-4108, K07-4020, and K07-4031, showed resistance to SBA similar to Jackson (Table 2-3).

**Table 2-4 Experiment 4: screening test for SBA resistance (15 lines)**

| Genotype | Total SBA number/plant | |
|---|---|---|
| K07-4073 | 47.6 | a† |
| K07-4026 | 33.6 | ab |
| K07-4023 | 24.6 | bc |
| KS4202 | 19.0 | bcd |
| K07-4085 | 10.5 | cde |
| K07-4065 | 4.7 | e |
| K07-4018 | 1.7 | e |
| K07-4063 | 1.4 | e |
| K07-4030 | 0.4 | e |
| K07-4076 | 0.4 | e |
| K07-4016 | 0.3 | e |
| K07-4067 | 0.2 | e |
| K07-4100 | 0.2 | e |
| K07-4108 | 0.2 | e |
| Jackson | 0.0 | e |
| K07-4020 | 0.0 | e |
| K07-4031 | 0.0 | e |
| LSD | 15.4 | |

† Mean aphid numbers followed by different letters are significantly different (P<0.05; t-test).

### *Experiment 5: screening test for SBA resistance (46 lines)*

A total of 46 Kansas soybean lines were screening for SBA resistance in 2010. The total aphid number on each genotype ranged from 0 to 58. Susceptible check KS4202 and resistant check Dowling had total aphid number of 27.8 and 0, respectively. Nineteen genotypes (bold in Table 2-5) showed resistance to SBA similar to Dowling.

**Table 2-5 Experiment 5: screening test for SBA resistance (46 lines)**

| Genotype | Total SBA number/plant | | Genotype | Total SBA number/plant | |
|---|---|---|---|---|---|
| K08-2532 | 58.0 | a† | K08-5764 | 12.5 | d-l |
| K08-5258 | 33.3 | b | K08-5597 | 12.5 | d-l |
| K08-6219 | 31.5 | bc | K03-3825 | 12.3 | d-l |
| K08-2509 | 31.3 | bc | **K08-6312** | 11.0 | e-l |
| K08-5760 | 31.0 | bc | **K04-3234** | 10.3 | f-l |
| K08-6336 | 28.0 | bcd | **K08-2454** | 10.0 | f-l |
| KS4202 | 27.8 | bcd | **K08-6591** | 9.3 | f-l |
| K08-5718 | 27.0 | b-e | **K08-1117** | 8.5 | g-l |
| K08-6406 | 25.3 | b-f | **K05-4184** | 8.0 | h-l |
| PIONEER 93M62 | 24.7 | b-g | **K08-5236** | 7.3 | h-l |
| K08-5208 | 22.0 | b-h | **5601T** | 6.7 | h-l |
| K08-5210 | 20.7 | b-i | **K08-5914** | 6.7 | h-l |
| ASGROW AG4403 | 20.5 | b-i | **LD00-281** | 6.5 | h-l |
| K08-5230 | 19.3 | b-j | **K08-6031** | 5.0 | i-l |
| K08-5472 | 19.3 | b-j | **K08-6374** | 5.0 | i-l |
| K08-5911 | 19.0 | b-j | **K08-5898** | 3.0 | jkl |
| K08-6263 | 17.0 | b-k | **KS5004N** | 2.3 | kl |
| K08-5125 | 16.3 | c-l | **K08-5892** | 2.0 | kl |
| K08-5570 | 16.0 | c-l | **K08-2449** | 1.3 | kl |
| K04-2203 | 14.3 | d-l | **K08-2043** | 1.3 | kl |
| K08-2529 | 14.0 | d-l | **K08-5984** | 1.0 | kl |
| K08-5862 | 13.3 | d-l | **Dowling** | 0.0 | l |
| K08-5241 | 13.3 | d-l | **K08-1692** | 0.0 | l |
| | | | LSD | 16.5 | |

† Mean aphid numbers followed by different letters are significantly different (P<0.05; t-test).

### *Experiment 6: screening test for SBA resistance (31 lines)*

A total of 31 Kansas soybean lines were screening for SBA resistance in 2010. The total aphid number on each genotype ranged from 0 to 28.5. Susceptible check KS4202 and resistant check Dowling had total aphid number of 25 and 0.6, respectively. Ten genotypes (bold in Table 2-5) showed resistance to SBA similar to Dowling.

**Table 2-6 Experiment 6: screening test for SBA resistance (31 lines)**

| Genotypes | Total SBA number/plant | | Genotype | Total SBA number/plant | |
|---|---|---|---|---|---|
| K03-3825 | 28.5 | a† | K09-1108 | 13.8 | c-h |
| K08-5349 | 27.8 | ab | K08-6238 | 13.0 | c-i |
| KS4202 | 25.0 | abc | K08-5864 | 13.0 | c-i |
| K08-6036 | 23.3 | a-d | K08-2452 | 13.0 | c-i |
| K05-2730 | 23.0 | a-d | K08-6247 | 12.8 | c-j |
| K08-6219 | 22.5 | a-d | **K08-6236** | 11.5 | d-k |
| K08-6067 | 21.5 | a-d | **K08-2545** | 11.3 | d-k |
| K08-5331 | 19.5 | a-e | **K08-6221** | 11.0 | d-k |
| K08-5286 | 19.0 | a-e | **K08-5863** | 8.8 | e-k |
| K09-1678 | 18.3 | a-f | **K09-3624** | 6.0 | f-k |
| K08-1170 | 17.0 | a-g | **K08-5760** | 5.3 | g-k |
| K08-2528 | 17.0 | a-g | **K08-5997** | 2.8 | h-k |
| IA3023 | 16.3 | a-g | **K09-3581** | 0.8 | ijk |
| K08-5764 | 15.8 | b-g | **Dowling** | 0.6 | jk |
| K09-3592 | 15.5 | b-g | **K09-2845** | 0.5 | jk |
| K08-2532 | 14.5 | c-h | **K09-1117** | 0.3 | k |
| K09-3584 | 14.5 | c-h | LSD | 12.4 | |

† Mean aphid numbers followed by different letters are significantly different (P<0.05; t-test).

## Conclusion and Discussion

A total of 150 Kansas soybean experimental lines have been screened for SBA resistance. Genotypes with resistance and moderate resistance have been discovered. K1639-2 has been released as germplasm resistant to SBA and soybean cyst nematode. K1639-2 and moderately resistant genotypes K1621, K1613, and K1642 were crossed with susceptible genotype KS4202 or resistant genotype Jackson or Dowling, to make several mapping populations. Study of $F_2$ generation of populations K1639-2/Jackson and K1639-2/Dowling indicated no segregation of SBA resistance among $F_2$ plants (Appendix A). K1639-2 might possess the same resistance gene as that in Dowling and Jackson. To discover new resistance gene, population of KS4202/K1621 will be used in further study. All of the identified resistant germplasm can be used in breeding program for SBA resistance.

# CHAPTER 3 - Inheritance of Soybean Aphid Resistance in Soybean Genotype K1621


## ABSTRACT

The soybean aphid (*Aphis glycines* Matsumura) has been a major threat to soybean [*Glycine max* (L.) Merr.] production in North America since its arrival in 2000. Host plant resistance is an effective method to control soybean aphid. Soybean genotype K1621 showed moderate antibiotic resistance against soybean aphid. The objective of this study was to determine the inheritance of soybean aphid resistance in K1621. Plants of $F_1$, $F_2$, and $F_2$-derived $F_3$ ($F_{2:3}$) families from a cross between K1621 and susceptible genotype KS4202 were evaluated for soybean aphid resistance. All $F_1$ plants showed resistance to the soybean aphid. Segregation of each of three independent $F_2$ populations and the pooled $F_2$ population data fit a single dominant gene ratio of 3:1 (P=0.28, 0.64, 0.26, and 0.85, respectively). The 150 $F_{2:3}$ families segregated in an expected 1:2:1 ratio. Our results indicated that one major dominant gene controls soybean aphid resistance in K1621. This gene may be useful in development of soybean cultivars that are resistant to soybean aphid.



*Key words*: Plant breeding, crop genetics, insect resistance, plant disease.


*Abbreviations*: SBA, soybean aphid; PI, plant introduction; IPM, integrated pest management.

# INTRODUCTION

Soybean aphid (*Aphis glycines* Matsumura) (SBA) has become an invasive pest insect to soybean [*Glycine max* (L.) Merr.] since its arrival in North America in 2000 (Hartman et al., 2001). The SBA causes damage by both direct feeding and transmitting virus diseases. Soybean plants can be infested by SBA at any growing stage and the worst damage occurs when plants are infested in the early seed-setting stage (Catangui et al., 2009). The SBA has spread to most major soybean- growing regions in the USA and some Canadian provinces (Venette and Ragsdale, 2004) and has been shown to cause significant yield losses in several Midwestern states (Ostlie, 2002; Rice et al., 2007; Steffey, 2004).

Compared with chemical and biological control, host plant resistance is an efficient, economical and environmentally friendly method for controlling SBA. In addition, plant resistance is an integral component of an integrated pest management (IPM) system (Auclair, 1989) and has been shown to be highly compatible with other control methods. Host plant resistance has three categories: antibiosis, antixenosis, and tolerance, and resistant plants may possess one or more of these categories (Painter, 1951; Smith, 2005).

Since the first report of SBA in North America, large numbers of soybean accessions have been screened for SBA resistance. Hill et al. (2004) identified seven soybean accessions with aphid resistance, including Dowling and Jackson. Further study showed that a single dominant gene controlled resistance in both Dowling (*Rag1*) and Jackson (Hill et al., 2006a, b). Mensah et al. (2005) identified four plant introductions (PI) with antixenotic resistance to SBA (PI 567598B, PI 567541B, PI567543C, and PI 567597C) and two with antibiotic resistance (PI 567598B and PI 567541B). Diaz-Montano et al. (2006) screened more than 200 commercial soybean lines and Kansas soybean experimental lines and identified Pioneer 95B97 and K1639

as highly antibiotic to SBA. Three other soybean genotypes (K1621, K1613, and K1642) showed moderate resistance. In 2006, a new biotype of SBA virulent to *Rag1* was identified and named the Ohio biotype (Kim et al., 2008). Screening for more SBA resistance sources and incorporating resistance genes into elite soybean varieties are tasks that need to be completed for breeders to contribute to effective management of SBA.

To develop durable SBA- resistant cultivars, the number and nature of resistance genes needs to be understood. Among resistant Kansas soybean lines, K1639 possesses the *Rag1* gene, but the moderately resistant K1621 does not. The objective of this study was to determine inherence of SBA resistance in the moderate resistance genotype K1621.

# MATERIALS AND METHODS

## *Population Development and $F_1$ Plant Evaluation*

Crosses were made between SBA-susceptible soybean genotype KS4202 and SBA-resistant genotype K1621 by transferring viable pollen from K1621 to the stigma of emasculated flowers of KS4202 under field conditions. KS4202 is the progeny from the cross of KS4694 /C1842, and K1621 is derived from the cross of NTCPR 94- 5483/'Pana'. The $F_1$ seeds were harvested and advanced to the $F_2$ generation. Seeds from selected $F_2$ plants were further advanced to $F_{2:3}$ families.

$F_1$ progeny of the cross were screened for resistance to the SBA Illinois biotype by using non-choice tests. The SBA used in all experiments were originally collected from a Kansas soybean field in Geary County, KS, in the summer of 2002. The SBA colony was maintained on soybean KS4202 plants in a growth chamber under a photoperiod of 14: 10(L: D) h and temperature of 24°C (day) and 20°C (night). $F_1$ plants, the susceptible (KS4202) and resistant (K1621) parents, and the resistant check Dowling were planted individually in 3.8-cm-diam. by 21.0-cm-deep plastic Cone-tainers (Ray Leach Cone-tainer, Hummert International, Earth City, MO) and arranged in a completely randomized design in a growth chamber under the same conditions described previously. Plastic trays were placed underneath the plants and filled with water to ensure each plant was watered evenly and consistently. When plants grew to V1 stage (Fehr and Caviness, 1977), two wingless aphid adults were transferred to the upper side of each unifoliolate leaf of each plant. Movement of aphids was restricted in double-sided sticky cages (Converters, Inc., Huntingdon Valley, PA) with an inner oval area of 1.2 $cm^2$. Cages were covered with a piece of mesh cloth right after aphids were put into the cages. The total number of aphids produced on each plant was counted 7d after infestation.

### *Screening of the $F_2$ Generation*

A total of 85 $F_2$ plants from three different $F_1$ plants were evaluated for SBA resistance following the same procedures described above. The $F_2$ plants, the KS4202 and K1621 parents, and the resistant check Dowling were planted in the same growth chamber under the same conditions used for $F_1$ evaluation. The number of SBA on each plant was counted 7d after infestation. Aphid numbers on resistant and susceptible parents were defined as *r* and *s*, respectively. The difference between *r* and *s* was defined as *d*. Any $F_2$ plants with aphid numbers $<(r+d/2)$ were considered resistant, and $F_2$ plants with aphid numbers $\geq(r+d/2)$ were considered susceptible. $F_2$ plants were classified as resistant when SBA numbers were <13, and lines were considered susceptible when aphid numbers were ≥13 at 7d after infestation. The goodness of fit of the observed segregation among all $F_2$ plants was performed using $\chi^2$ tests.

### *Screening of $F_{2:3}$ Families*

A population of 150 $F_{2:3}$ soybean families was evaluated for SBA resistance. Each $F_3$ family contained up to 22 plants that were grown individually along with the resistant (K1621) and susceptible (KS4202) parents in Cone-tainers and arranged in a randomized complete block design with three replicates in the greenhouse under a photoperiod of 14:10(L:D) h and temperature of 20 to 28°C. The greenhouse screening procedures were the same as those described for the $F_1$ and $F_2$ screenings in the growth chamber. Each plant in this experiment was evaluated for aphid resistance. The $F_{2:3}$ lines were classified according to the $(r+d/2)$ calculation as resistant when SBA numbers were <20, and lines were considered susceptible when aphid numbers were ≥20 at 7d after infestation. The $F_{2:3}$ families were classified as homozygous resistant if all plants in a family were resistant, homozygous susceptible if all plants in a family

were susceptible, or heterozygous if both resistant and susceptible plants in a family were identified. The goodness of fit among $F_{2:3}$ families was performed using $\chi^2$ tests.

# RESULTS

## *$F_1$ Generation Plants*

The total number of SBA produced on K1621 ranged from 0 to 14 with an average of 7.4; the total number of SBA on KS4202 ranged from16 to 42 with an average of 25.9 (Table 1). Among the eight $F_1$ plants screened for SBA resistance, the number of aphids per plant ranged from 5.5 to 10 with an average of 7.9 aphids. Phenotypic damage ratings of the $F_1$ plants were not significantly different from ratings of K1621 (P=0.90) but were significantly lower than ratings for the susceptible control KS4202 (P<0.0001).

**Table 3-1 Mean number of soybean aphids on Dowling (resistant control), K1621 (resistant), KS4202 (susceptible), and an $F_1$ population derived from the cross of K1621 and KS4202 at 7d after infestation.**

| Soybean genotype | Number of plants | Mean ± S.E. number of soybean aphids per plant† |
|---|---|---|
| KS4202 | 10 | 25.9±2.4a |
| K1621 | 7 | 7.4±2.9bc |
| F1 | 8 | 7.9±2.7b |
| Dowling | 7 | 0±2.9c |

† Mean aphid numbers followed by different letters are significantly different (P<0.05; t-test).

### *$F_2$ Generation Plants*

The total number of SBA on $F_2$ plants ranged from 0 to 29, and the difference between the average numbers of SBA on resistant K1621 plants (5.3) and susceptible KS4202 (22.3) was considerable (17). Each of the three $F_2$ populations segregated in a 3:1 resistant/susceptible ratio (P=0.28, 0.64, and 0.26, respectively) (Table 2). Segregation of the overall $F_2$ progeny fit the 3:1 resistant/susceptible ratio (P=0.85), indicating that a single dominant gene controls SBA resistance in K1621.

**Table 3-2 Segregation of 85 $F_2$ plants in three $F_{1:2}$ families for resistance to soybean aphid in a population from the cross of K1621 (resistant) and KS4202 (susceptible) at 7d after infestation.**

| | Number | Observed† | | Expected (3:1) | | | |
|---|---|---|---|---|---|---|---|
| $F_{1:2}$ family | of plants | Resistant | Susceptible | Resistant | Susceptible | $\chi^2$ | P |
| 1 | 35 | 29 | 6 | 26.3 | 8.8 | 1.15 | 0.28 |
| 2 | 24 | 17 | 7 | 18 | 6 | 0.22 | 0.64 |
| 3 | 26 | 17 | 9 | 19.5 | 6.5 | 1.28 | 0.26 |
| Pooled | 85 | 63 | 22 | 63.8 | 21.3 | 0.04 | 0.85 |

† Resistant= total aphid number per plant<13; Susceptible= total aphid number per plant≥13.

### *$F_{2:3}$ Families*

K1621 and KS4202 had total SBA numbers of 11.9 (resistant) and 27.9 (susceptible), respectively, at 7d after infestation, and the difference (16) between the parents was similar to that observed in the previous experiments with $F_1$ and $F_2$ plants. Segregation among $F_{2:3}$ families for SBA resistance fit a 1:2:1 resistant/heterozygous/susceptible ratio (P=0.09; Table 3). The segregation ratios of the $F_2$ plants and $F_{2:3}$ families indicated that SBA resistance in K1621 is controlled by a major dominant gene. Noticeably, K1621 and KS4202 sustained greater SBA reproduction in this experiment when grown with the $F_3$ generation plants than with either the $F_1$ or $F_2$ generation plants. This is because the $F_{2:3}$ families were evaluated under a different environment than the $F_1$ and $F_2$ generations. However, the consistent difference, *d*, indicated the resistance evaluation method was reliable under different environments.

**Table 3-3 Segregation of 150 $F_{2:3}$ families from the cross of K1621 (resistant) and KS4202 (susceptible) for resistance to the soybean aphid at 7d after infestation.**

| Number of | Observed† | | | Expected (1:2:1) | | | | |
|---|---|---|---|---|---|---|---|---|
| $F_{2:3}$ families | R | H | S | R | H | S | χ2 | P |
| 150 | 33 | 88 | 29 | 37.5 | 75 | 37.5 | 4.72 | 0.09 |

† R(resistant)= all plants in an F2:3 family were resistant if the number of aphids per plant was <20; H (heterozygote)= plants in a family segregated for resistance; S= all plants in an F2:3 family were susceptible if the number of aphids per plant was ≥20.

# DISCUSSION

Two SBA resistance genes have been identified and named. *Rag1* from Dowling is resistant to the SBA Illinois biotype (Hill et al., 2006a; Li et al., 2007) and *Rag2* from PI 243540 is resistant to the SBA Ohio biotype (Kang et al., 2008; Mian et al., 2008). The relationships between the resistance gene in K1621 identified in this study and *Rag1* and *Rag2* are not known. Because this gene controls resistance to the Illinois biotype, K1621 might be a new resource for breeding SBA-resistant soybean. Phenotypic expression in the $F_1$ and segregation in the $F_2$ and $F_{2:3}$ generations derived from the cross between resistant genotype K1621 and susceptible genotype KS4202 indicated that a single dominant gene controls SBA resistance. However, compared with the highly resistant cultivars Dowling and Jackson, K1621 possess moderate SBA resistance.

Other SBA resistance resources include two recessive genes in PI567541B and PI567598B (Mensah et al., 2008; Zhang et al., 2009) and a dominant gene from PI200538 that confers antixenotic resistance to both the Illinois and Ohio biotypes (Hill et al., 2009). These genes are unnamed, and their allelism with the gene in K1621 is unknown.

Aphid number is a good indicator of plant antibiotic resistance because antibiosis affects the biology and population development of the insect. The screening method used in this study is a simple, quick, and efficient way to evaluate soybean germplasm for resistance to SBA. This method requires inclusion of resistant and susceptible checks in every screening test to compensate for the effects of environmental variation. Aphid population counts are also a good way to measure a trait that is not easily classified as either resistant or susceptible. In addition, an aphid population count is a trait that can be measured as a continuous number and easily used in

quantitative trait loci (QTL) mapping studies to further investigate the inheritance of resistance genes such as the dominant trait in K1621.

The dominant inheritance of SBA resistance in K1621 will be useful for introgression of the gene controlling resistance into adapted, elite soybean breeding lines. A goal of future research will be the identification and development of DNA markers tightly linked with the resistance gene in K1621. Marker-assisted selection can then be used to facilitate incorporation of the K1621 resistance gene into elite cultivars in a more rapid and efficient manner.

# REFERENCES


Auclair, J.L. 1989. Host plant resistance. p. 225–265. *In* A.K. Minks and P. Harrewijn (ed.) Aphids: Their biology, natural enemies, and control. Vol. C. Elsevier, New York.

Catangui, M.A., E.A. Beckendorf, and W.E. Riedell. 2009. Soybean aphid population dynamics, soybean yield loss, and development of stage-specific economic injury levels. Agron. J. 101:1080-1092.

Diaz-Montano, J., J.C. Reese, W.T. Schapaugh, and L.R. Campbell. 2006. Characterization of antibiosis and antixenosis to the soybean aphid (Hemiptera: Aphididae) in several soybean genotypes. J. Econ. Entomol. 99(5): 1884-1889.

Fehr, W.R., and C.E. Caviness. 1977. Stages of soybean development. Spec. Rep. 80. Iowa Agric. Home Econ. Exp. Stn., Iowa State Univ., Ames.

Hartman, G.L., L.L. Domier, L.M. Wax, C.G. Helm, D.W. Onstad, J.T. Shaw, L.F. Solter, D.J. Voegtlin, C.J. D'Arcy, M.E. Gray, K.L. Steff ey, S.A. Isard, and P.L. Orwick. 2001. Occurrence and distribution of *Aphis glycines* on soybeans in Illinois in 2000 and its potential control. Plant Health Progress doi:10.1094/PHP-2001-0205-01-HN.

Hill, C.B., K.S. Kim, L. Crull, B.W. Dier, and G.L. Hartman. 2009. Inheritance of resistance to the soybean aphid in soybean PI 200538. Crop Sci. 49:1193-1200.

Hill, C.B., Y. Li, and G.L. Hartman. 2004. Resistance to the soybean aphid in soybean germplasm. Crop Sci. 44:98-106.

Hill, C.B., Y. Li, and G.L. Hartman. 2006a. A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. Crop Sci. 46:1601-1605.

Hill, C.B., Y. Li, and G.L. Hartman. 2006b. Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. Crop Sci. 46:1606-1608.

Kang, S.T., M.A. R. Mian, and R.B. Hammond. 2008. Soybean aphid resistance in PI 243540 is controlled by a single dominant Gene. Crop Sci. 48:1744-1748.

Kim, K.S., C.B. Hill, G.L. Hartman, M.A.R. Mian, and B.W. Diers. 2008. Discovery of soybean aphid biotypes. Crop Sci. 48:923–928.

Li, Y., C.B. Hill, S.R. Carlson, B.W. Diers, and G.L. Hartman. 2007. Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group M. Mol. Breeding 19:25-34.

Mensah, C., C. DiFonzo, R.L. Nelson, and D. Wang. 2005. Resistance to soybean aphid in early maturing soybean germplasm. Crop Sci. 45:2228-2233.

Mensah, C, C. Difonzo, and D. Wang. 2008. Inheritance of soybean aphid resistance in PI 567541B and PI 567598B. Crop Sci. 48:1759-1763.

Mian, M.A.R., S.T. Kang, S.E. Beil, and R.B. Hammond. 2008. Genetic linkage mapping of the soybean aphid resistance gene in PI 243540. Theor. Appl. Genet. 117:955-962.

Ostlie, K. 2002. Managing soybean aphid. Univ. of Minnesota Extension Serv, St Paul. http://www.soybeans.umn.edu/crop/insects/aphid/aphid_publication_managingsba.htm.

Painter, R.H. 1951. Insect resistance in crop plants. MacMillan Co., New York.

Rice, M.E., M. O'Neal, P. Pedersen. 2007. Soybean aphids in Iowa-2007. Iowa State Univ. University Extension. Available at http://extension.agron.iastate.edu/soybean/documents/SP247Aphids07.pdf

Smith, C.M. 2005. Plant resistance to arthropods: molecular and conventional approaches. Springer, The Netherlands.

Steffey, K.L. 2004. Soybean aphids in Illinois [Online]. Available at www.ipm.uiuc.edu/fieldcrops/insects/soybean_aphids/workshop/SoybeanAphidsInIllinois_Steffey.ppt (verified 18 Mar. 2006). Univ. of Illinois at Urbana-Champaign.

Venette, R.C. and D.W. Ragsdale. 2004. Assessing the invasion by soybean aphid (Homoptera: Aphididae): Where will it end? Ann. Entomol. Soc. Am. 97: 219D226.

Zhang, G., C. Gu, and D. Wang. 2009. Molecular mapping of soybean aphid resistance genes in PI567541B. Theor. Appl. Genet. 118:473-482.

# CHAPTER 4 - QTL Mapping of Soybean Aphid Resistance Gene in K1621

## ABSTRACT

The soybean aphid (*Aphis glycines* Matsumura) has been one of the major pests of soybean [*Glycine max* (L.) Merr.] in soybean-growing regions of North America since it was first reported in 2000. K1621 is a soybean genotype with moderate resistance to soybean aphid biotype 1. The objectives of this study were to map and validate the resistance gene in K1621 using simple sequence repeat (SSR) markers. A mapping population of 150 $F_{2:3}$ families from the cross between K1621 and susceptible genotype KS4202 were evaluated for aphid resistance. Phenotyping was conducted on the basis of total aphid number per plant 7 days following infestation with 4 aphids. After SSR markers for polymorphism were screened between parents, a total of 133 polymorphic markers distributed across the soybean genome were used for genotyping. One quantitative trait loci (QTL) controlling antibiotic resistance was found by using the composite interval mapping method. This QTL localized on chromosome 13 (linkage group F) between markers Sat_234 and S6814 and explained 54% of the phenotypic variation. The putative QTL was further validated by single marker analysis using an independent population derived from the cross of K1621 and Dowling. The locus for soybean aphid resistance in K1621 was named [*Rag*]_K1621. The markers identified and validated in this study could be useful for marker-assisted selection of [*Rag*]_K1621.

*Key words:* Plant breeding, crop genetics, insect resistance, plant disease.

*Abbreviations*: SBA, soybean aphid; QTL, quantitative trait loci; LG, linkage group; PI, plant introduction; SSR, simple sequence repeat; CIM, composite interval mapping; SCN, soybean cyst nematode.

# INTRODUCTION

The soybean aphid (*Aphis glycines* Matsumura) (SBA) is one of the most serious threats to soybean [*Glycine max* (L.) Merr.] production in China and other Asian countries (Wu et al. 2004). Since the SBA was introduced to North America in 2000 (Hartman et al. 2001), it has spread to most major soybean-growing regions in the United States and some areas in Canada (Venette and Ragsdale 2004). The SBA causes damage by direct feeding and transmitting virus diseases. Damage can occur at any soybean growth stage, but the worst damage is caused by infestation at the pod setting stage. Yield loss caused by heavy SBA infestation was 32% in Iowa in 2003 (Rice et al. 2007) and more than 50% in Minnesota (Ostlie 2002), and can be up to 50-70% in China (He et al. 1991).

A supplement to chemical and biological control, plant resistance is an efficient, economical, and environmentally friendly method to control SBA. A large amount of soybean germplasm has been screened for SBA resistance, and a few resistant genotypes have been identified (Diaz-Montano et al. 2006; Hill et al. 2004; Mensah et al. 2005; Mian et al. 2008a). Further studies showed that resistant varieties Dowling and Jackson possess a single dominant gene to SBA biotype 1 (Hill et al. 2010), and both genes were mapped to the same region in chromosome 7 [linkage group (LG) M] (Hill et al. 2006a, b). The resistance gene in Dowling was named *Rag1,* and that in Jackson remained unnamed because of its unknown relationship with *Rag1* (Li et al. 2007). Shortly after *Rag1*was reported, a new biotype of soybean aphid (biotype 2) was identified and believed to be virulent to *Rag1* (Kim et al. 2008; Hill et al. 2010). Later, a resistance gene to SBA biotype 2 was mapped to chromosome 13 (LG F) and named *Rag2* (Mian et al. 2008b). The third SBA resistance gene *Rag3* was mapped to chromosome 16

(LG J) by Zhang et al. (2010). Two quantitative trait loci (QTL) associated with SBA resistance in early maturing germplasm PI567541B were mapped to chromosome 7 and 13 (LG M and F; Zhang et al. 2009). More recently, a single dominant gene controlling resistance to both biotypes in PI200538 was mapped to chromosome 13 (LG F; Hill et al. 2009) in the same region as *Rag2*. The report of new SBA biotype (biotype 3) in 2010 (Hill et al. 2010) made the task of soybean breeding for aphid resistance more challenge.

Diaz-Montano et al. (2006) identified some Kansas soybean experimental lines, including K1621, with moderate resistance to the SBA biotype 1. Further study of the inheritance of SBA resistance indicated that a single dominant gene controls resistance in K1621 (Meng et al. 2010). Therefore, objectives of this study were to map the soybean aphid resistance gene in K1621 using simple sequence repeat (SSR) markers and validate the identified gene with another independent population.

# MATERIALS AND METHODS

## *Soybean aphid culture*

Soybean aphids were originally collected from a soybean field in Geary County, Kansas, in summer 2002. The SBA colony is maintained on soybean KS4202 plants in a growth chamber under a photoperiod of 14 h light: 10 h dark and temperature of 24°C (day) and 20°C (night). The aphid colony is the biotype 1 and virulent to KS4202 (Dr. John Reese, personal communication).

## *Population development*

Two separate populations were used in this study. The first population (mapping population) was developed by crossing soybean genotypes KS4202 and K1621 and advancing the progeny to the $F_{2:3}$ generation. In addition, K1621 was crossed to Dowling and the $F_1$ seeds were harvested and advanced to the $F_{2:4}$ generation to form the second population (validation population). KS4202 is susceptible to soybean aphids, K1621 has moderate resistance to SBA biotype 1, and Dowling contains the *Rag1* gene, which is also resistant to SBA biotype 1.

## *Aphid resistance evaluation*

No-choice tests were conducted to evaluate plant antibiotic resistance in the greenhouse under a photoperiod of 14 h light: 10 h dark and temperature of 20-28°C. The first population of 150 $F_{2:3}$ families and parents, KS4202 and K1621, was grown individually in plastic Cone-tainers (3.8-cm diameter by 21.0-cm deep, Ray Leach Cone-tainer, Hummert International, Earth City, MO) arranged in randomized complete block design with 3 replicates. Each family

contained up to 22 $F_3$ plants across the replications. Plastic trays were placed underneath the plants and filled with water to assure each plant was watered evenly and consistently.

Each plant was infested with two wingless SBA adults on the upper side of each unifoliolate leaf at V1 stage. Movement of SBA was restricted in double-side sticky cages (Converters, Inc., Huntingdon Valley, PA) with an inner oval area of 1.2 $cm^2$. Cages were covered with a piece of mesh cloth immediately after aphids were placed into the cages. Soybean aphid resistance was evaluated by counting the total number of aphids on each plant 7 days following infestation.

To validate the identified QTL, the second population of 106 $F_{2:4}$ families and parents, K1621 and Dowling, was phenotyped following the procedure mentioned previously. Each family contained up to 14 $F_4$ plants across the replications. Experiments were arranged in a randomized complete block design with two replicates in the greenhouse under the same environmental conditions as the first population.

### *DNA extraction and marker analysis*

Non-expanded trifoliolate leaves from each line were bulk harvested for use in isolating genomic DNA using the CTAB (hexadecyltrimethyl ammonium bromide) method (Gill et al. 1991). The DNA samples were quantified with a NanoDrop™ 1000 spectrophotometer (Thermo Scientific, Wilmington, DE) and diluted to 10-50 ng $\mu l^{-1}$ for genotyping. A total of 543 SSR markers covering the soybean genome (Song et al. 2004) were screened for polymorphism between parents. The SSR marker sequences were obtained from soybase.org or provided by Dr.

Perry Cregan at USDA-ARS, Beltsville, MD. PCR reactions were done in 12 µl volumes with 20-100 ng of template DNA, 10 µM of each primer, and 1X PCR Master Mix (Promega, Madison, WI; including 0.25 U of Taq DNA polymerase, 200 µM each of dNTP, and 1.5mM $MgCL_2$). Reactions were run on a PTC100 Thermal Cycler (MJ Research INC., Watertown, MA) using the thermal profile consisting of an initial denaturing step of 94°C for 1 min followed by 32 cycles of 25 s of denaturing at 92°C, 25 s of annealing at 47°C, 25 s of extension at 68°C, and a final 10 min extension at 68°C. Amplified products were separated on 2% agarose gels in 1x TAE buffer. Gels were stained with ethidium bromide, visualized under UV light, and recorded with an AMBIS Radioanalytic Imaging System (Digital Imagers, Madison, WI).

Polymorphic markers were used to genotype the mapping population with an ABI 3730 sequencer (Applied Biosystems Inc, Foster City, CA). The forward primer of each pair of markers was modified by adding an 'M13' tail to the 5' end with the sequence of 5'-ACG ACG TTG TAA AAC GAC-3'. Each marker was labeled with fluorescent dye FAM, VIC, PET or NED in PCR reactions. A 12-µl PCR mixture included 20-100 ng of template DNA, 100nM forward tailed primer, 200nM reverse primer, 100nM M13 dye-labeled primer, and 1X PCR Master Mix. PCR amplification used a touch-down program starting with 5 min of denaturing at 95°C followed by 30 s at 96°C, 30 s at 47°C, 30 s at 72°C with a 0.5°C decrease of annealing temperature in each of the following 13 cycles, and 30 cycles of 30 s at 96°C, 30 s at 40°C, and 30 s at 72°C with a final extension of 5 min at 72°C. PCR products with different dyes were pooled proportionally and separated with an ABI 3730 sequencer. Amplification results were visualized and scored with GeneMarker 1.7 (Softgenetics LLC, State College, PA).

### *Statistical and QTL analysis*

An ANOVA was performed using SAS Proc GLM (SAS 9.1, Cary, NC) to analyze phenotypic data. The broad sense heritability of aphid number was calculated as $h^2 = (\sigma^2_{phF2} - \sigma^2_e)/ \sigma^2_{phF2}$. The genetic linkage map was constructed using MapMaker 3.0 with the Kosambi function. The threshold LOD score started with 3.0 to build up initial linkage groups and then decreased to 2.0 to include additional markers. Single marker analysis (SMA) and composite interval mapping (CIM) were performed using QTLCartorgrapher2.5 with the standard model Zmapqtl6 (Wang et al. 2008). The window size for background control was set to 5cM. The forward and backward regression method was used to select five markers as cofactors. The walking speed was 2cM. The threshold LOD score at 5% probability level was determined by a permutation test with 1,000 repetitions.

### *QTL validation*

The validation population was used to confirm the aphid resistance QTL identified in the mapping population and to test for interaction with *Rag1*. Phenotyping and genotyping were conducted following the previously described procedures. Markers used for validation were chosen from linkage groups in which the identified aphid resistance QTL and *Rag1* are located. Markers that were significant in the single marker analysis were further investigated using SAS PROC GLM with the model of $Y= a+bX_1+cX_2+dX_1X_2+e$, where Y is the phenotypic performance, and b, c, and d are the effects of two markers from each linkage group.

### *Ancestry study*

The pedigree of K1621 was examined. Nineteen of the ancestors of K1621 were introductions from other countries and the common ancestors of most soybean cultivars in North America (Table 2; Allen and Bhardwaj 1987). Markers tightly linked to SBA resistance in both populations were used to genotype the 19 ancestors, KS4202, K1621, and Dowling. The PCR products were separated on 3% agarose gels in 1x TAE buffer and visualized under UV light. Ancestral genotypes that have the same allele as K1621 at the markers' loci might be contributors of the SBA resistance in K1621.

# RESULTS

## *Aphid resistance evaluation*

Total aphid numbers were significantly ($P<0.05$) different among the $F_{2:3}$ lines, ranging from 6 to 43 aphids per plant. Parents K1621 and KS4202 had aphid numbers of 11 and 29, respectively. The frequency distribution of aphid number was continuous and normal (Fig. 1), indicating there might be more than one gene controlling the aphid resistance. Broad sense heritability was 60%.

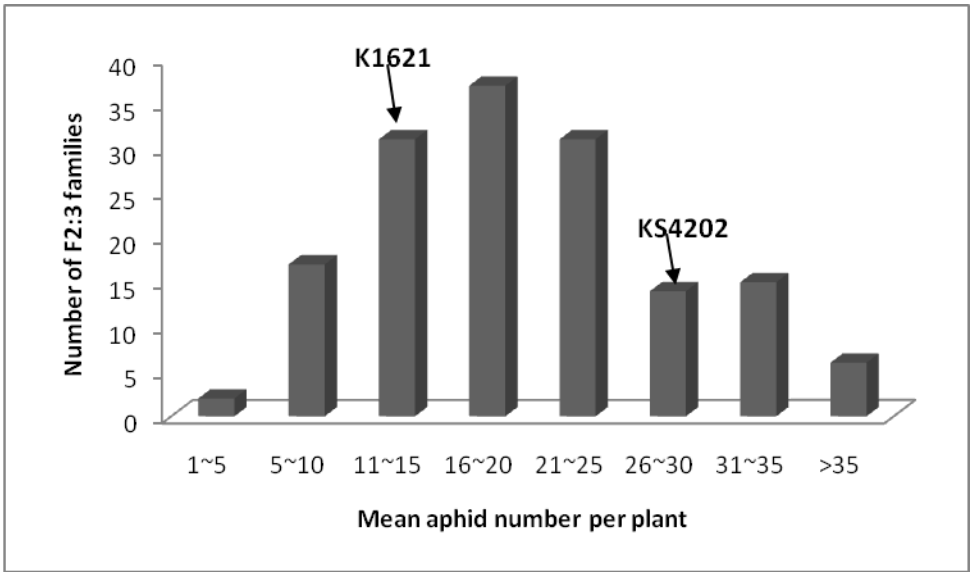



**Figure 4.1 Frequency distribution of soybean aphid number per plant for 150 $F_{2:3}$ families derived from the cross of KS4202 and K1621.**

Arrows indicate parents.

### *QTL mapping*

Out of 543 screened SSR markers, 133 markers spanning all soybean linkage groups were polymorphic between the two parents and used for linkage mapping. These markers were mapped into 28 linkage groups, some of which were fragments of the same linkage group on the consensus map (Song et al. 2004). The linkage map generated in this study was consistent with the consensus map in terms of marker orders. Distances between markers were about two times longer than those in the consensus map because of the limited population size and marker scoring errors. The total map length was 1,647.6 cM with an average interval length of 14.6 cM between two markers. This map was used to map QTL. Single marker analysis indicated a cluster of markers on chromosome 13 (LG F) was highly significantly related to the potential QTL. Composite interval mapping revealed one QTL with a LOD score of 18.06 on chromosome 13 (LG F) with the peak position 2.8 cM away from marker S6814 and 6.1 cM away from marker Sat_234 (Fig. 2). This QTL explained 54% of the phenotypic variation in aphid number.

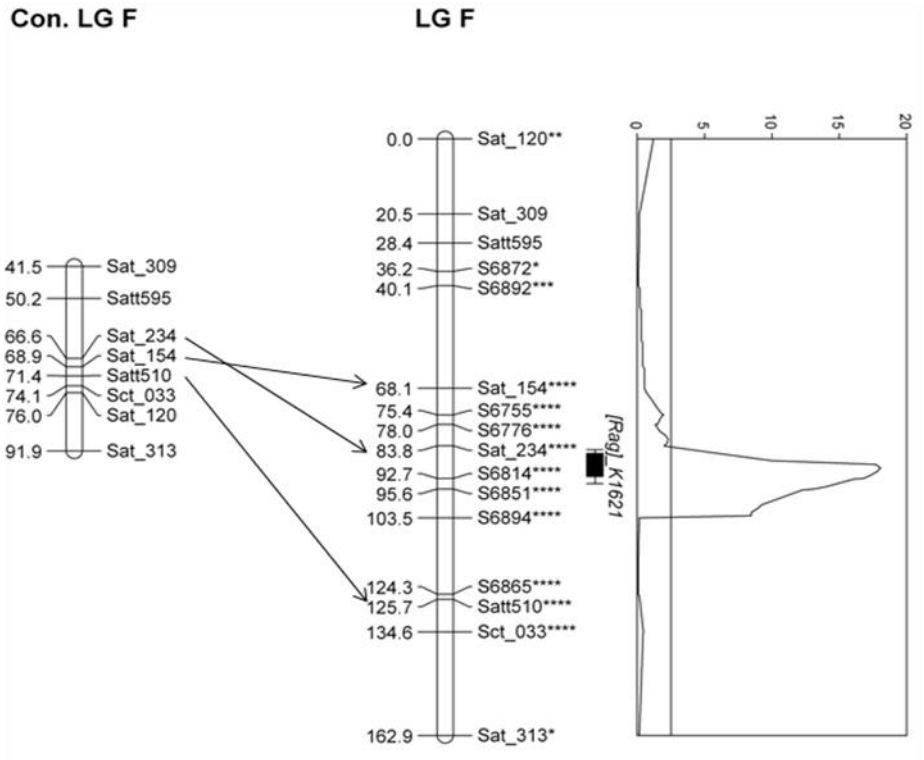



**Figure 4.2 Corresponding segments of consensus soybean linkage group F (Con. LG F) and linkage group F (LG F) constructed in this study.**

The location of the soybean aphid resistance QTL was identified using the composite interval mapping method. 1-LOD and 2-LOD support intervals of QTL are marked by thick and thin bars respectively. Asterisks indicate significance level of markers in single marker analysis (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

### *QTL validation*

Phenotyping of the validation population showed that *Rag1* had a strong effect on aphid resistance, indicated by the skewed frequency distribution of aphid number among the 106 $F_{2:4}$ families. The fact that K1621 had a total aphid number of 11 and 26 in two different populations demonstrated the environmental effect of the QTL in K1621 (Fig. 3). Single marker analysis confirmed that four markers, including previously identified linked marker S6814, are closely linked to the QTL on chromosome 13 (LG F; Table 1). Unfortunately, Sat_234 was not polymorphic between Dowling and K1621. The QTL on chromosome 13 (LG F) and *Rag1* explained 6% and 47% phenotypic variation, respectively. Closely linked markers S6814 in chromosome 13 (LG F) and *Rag1* linked marker Satt435 were tested for interaction in the same linear model. Results showed there was no interaction between markers S6814 and Satt435 (P=0.37). Zhang et al. (2009) detected two QTLs in chromosomes 13 (LG F) and chromosome 7 (LG M) at different loci from the QTL identified in this study. They also detected significant interaction between these two QTLs. The locus for SBA resistance in K1621 has been named [*Rag*]_K1621.

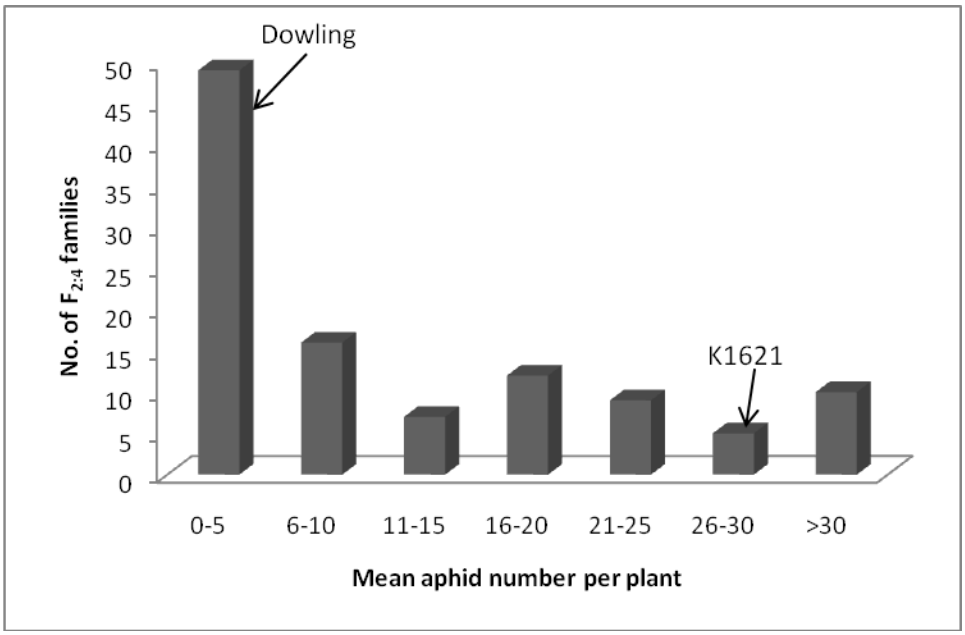



**Figure 4.3 Frequency distribution of soybean aphid number per plant for 106 $F_{2:4}$ families derived from the cross of K1621 and Dowling.**

Arrows indicate parents.

**Table 4-1 Single marker analysis of validation population.**

| Marker | Linkage Group | Test Statistic[a] | F value | pr(F) | |
|---|---|---|---|---|---|
| Satt490 | F | 3.262 | 3.25 | 0.074 | |
| Sat_154 | F | 6 | 6.057 | 0.015 | * |
| S6814 | F | 16.162 | 17.129 | <0.0001 | **** |
| S6755 | F | 11.845 | 12.296 | 0.001 | *** |
| S6776 | F | 12.55 | 13.072 | <0.001 | *** |
| Satt663 | F | 1.515 | 1.497 | 0.224 | |
| Satt516 | F | 0.135 | 0.132 | 0.717 | |
| Sat_240 | F | 0.142 | 0.139 | 0.710 | |
| Sat_298 | F | 0.004 | 0.004 | 0.952 | |
| BE806387 | F | 0.034 | 0.033 | 0.856 | |
| Satt659 | F | 0.187 | 0.184 | 0.669 | |
| S6865 | F | 3.152 | 3.139 | 0.079 | |
| Sat_112 | F | 0.534 | 0.525 | 0.470 | |
| Satt395 | F | 0.672 | 0.661 | 0.418 | |
| Satt656 | F | 0.006 | 0.006 | 0.938 | |
| Satt175 | M | 19.721 | 21.266 | <0.0001 | **** |
| Satt626 | M | 24.037 | 26.471 | <0.0001 | **** |
| Satt323 | M | 22.946 | 25.136 | <0.0001 | **** |
| Satt220 | M | 20.127 | 21.747 | <0.0001 | **** |
| Satt245 | M | 41.983 | 50.54 | <0.0001 | **** |
| Satt463 | M | 62.522 | 83.582 | <0.0001 | **** |
| Satt435 | M | 74.232 | 105.494 | <0.0001 | **** |
| Satt540 | M | 82.259 | 121.974 | <0.0001 | **** |

[a] Likelihood ratio test statistic is -2ln(L0/L1), where L0 is the likelihood of no gene effect and L1 is the likelihood of gene effect. Asterisks indicate significance level of marker in single marker analysis (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

### *Ancestry study*

KS4202, K1621, Dowling, and 19 ancestors of K1621 were genotyped with markers S6814, S6755, and S6776. All three markers were closely linked to [*Rag*]_K1621. KS4202, K1621, and Dowling have different alleles of each marker (Table 2). At the loci of S6814, S6755, and S6776, there are 5, 5, and 4 of the 19 ancestors that have same allele as K1621. Ancestor Palmetto (PI 548480) has the same allele as K1621 for all three markers, indicating that SBA resistance in K1621 might be inherited from Palmetto. In non-choice tests, the SBA resistance effect in Palmetto was similar to that in Dowling and slightly stronger than that in K1621 (Diaz-Montano 2006; Hill et al. 2004).

**Table 4-2 List of selected ancestors of K1621 and their alleles of marker S6814, S6755, and S6776.**

| PI | Cultivar name | S6814 | | | S6755 | | | S6776 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | KS4202 289bp | K1621 300bp | Dowling 286bp | KS4202 294bp | K1621 297bp | Dowling 282bp | KS4202 143bp | K1621 135bp | Dowling 150bp |
| 548298 | A.K. (Harrow) | | | + | + | | | + | | |
| 548438 | Arksoy | | | | | + | | + | | |
| 548445 | CNS | | | | + | | | | | + |
| 548318 | Dunfield | | | | + | | | | + | |
| 548456 | Haberlandt | | | | | + | | | | + |
| 548348 | Illini | | + | | + | | | + | | |
| 548379 | Mandarin(Ottwa) | | | | | | + | | + | |
| 548391 | Mukden | + | | | | | + | + | | |
| 548477 | Ogden | | + | | | | + | + | | |
| 548480 | Palmetto | | + | | | + | | | + | |
| 548400 | Patoka | | | + | + | | | + | | |
| 548484 | Ralsoy | | | | | + | | | | + |
| 548406 | Richland | | | | + | | | + | | |
| 548485 | Roanoke | + | | | | | + | | | + |
| 548488 | S-100 | | + | | + | | | + | | |
| 548493 | Tokyo | + | | | | + | | + | | |
| 88788 | | | | | + | | | | + | |
| 438497 | Peking | | + | | + | | | | | + |
| 54610 | | + | | | | | + | | | + |

+ Presence of corresponding alleles of each mark

# DISCUSSION

A major QTL controlling moderate SBA resistance in K1621 was identified in this study. This QTL was further confirmed using an independent population. The QTL was mapped to chromosome 13 (LG F) between SSR markers Sat_234 and S6814 and explained a large portion of the phenotypic variation. These two markers are less than 5 cM apart on the consensus map (Song et al. 2004) and about 9 cM apart on the genetic map generated in this study. This anomaly is not unexpected and could be the result of the small population size and different population structure used in this study. The SBA resistance gene *Rag2* from PI 243540 that confers resistance to SBA biotype 2 was mapped to chromosome 13 (LG F; Mian et al. 2008b) between markers Satt334 and Sct_033, which are 12.1 and 7.6 cM, respectively, away from Sat_234. Though the QTL identified in our study controls resistance to SBA biotype 1, it could be a new allele at *Rag2* locus or a new gene. Hill et al. (2009) also identified a SBA gene from PI 200538 in the same region within the interval of markers Sat_234 and Satt510 that confers antixenotic resistance to both biotypes 1 and 2. Fine mapping of *Rag2* from PI 200538 narrowed the gene down to a 54-kd region (Kim et al. 2010). Nevertheless, neither PI 243540 nor PI 200538 is in the pedigree of K1621. The QTL identified in this study has a fairly small interval, and the flanking markers can be used for marker-assisted selection.

The region ±10 cM from Sat_234 is a hot spot for disease resistance genes. The *Rpg1* gene that controls resistance to bacterial blight (*Pseudomonas syringae*) was mapped in the same interval as [*Rag*]_K1621 (Ashfield et al. 1998). Other genes in this R-gene cluster region include *Rpv1* for resistance to peanut mottle virus (Gore et al. 2002), *Rsv1* for resistance to soybean mosaic virus (Gore et al. 2002; Yu et al. 1994) and *Rps3* for resistance to *Phytophthora sojae*

(Demirbas et al. 2001; Diers et al. 1992). Interestingly, some QTLs correlated with biotic stress resistance are also located in this region, including CEW2-1(corn earworm resistance; Rector et al. 1999; 2000), Ma1-2, 1-4, 1-5, 2-1(peanut root-knot nematode resistance; Tamulonis et al. 1997a), and Mj1-4, 1-6, 2-1(Javanese root-knot nematode resistance; Mienie et al. 2002; Tamulonis et al. 1997b). The QTLs related to nematodes are of particular interest because the first cloned potato aphid (*Macrosiphum euphorbiae*) resistance gene in tomato, *Mi-1.2*, also confers resistance to root-knot nematode (*Meloidogyne incognita*; Rossi et al. 1998). K1621 is also resistant to soybean cyst nematode (SCN; *Heterodera glycines*), one of the most severe soybean pathogens in North America. There are three QTLs controlling resistance to SCN mapped to different regions on chromosome 13 (LG F; reviewed by Concibido et al. 2004). K1621 can serve as useful germplasm in breeding programs for both soybean aphid and nematode resistance.

# REFERENCES


Allen FL, Bhardwaj HL (1987) Genetics relationships and selected pedigree diagrams of North American soybean cultivars. The University of Tennessee Agricultural Experiment Station

Ashfield T, Danzer JR, Held D, Clayton K, Keim P, Saghai Maroof MA, Webb DM, Innes RW (1998) *Rpg1*, a soybean gene eVective against races of bacterial blight, maps to a cluster of previously identified disease resistance genes. Theor Appl Genet 96:1013–1021

Concibido VC, Diers BW, Arelli PR (2004) A decade of QTL mapping for cyst nematode resistance in soybean. Crop Sci 44: 1121-1131

Demirbas A, Rector BG, Lohnes DG, Fioritto RJ, Graef GL, Cregan PB, Shoemaker RC, Specht JE (2001) Simple sequence repeat markers linked to the soybean rps genes for phytophthora resistance. Crop Sci 41:1220–1227

Diaz-Montano J (2006) Components of soybean resistance to the soybean aphid, *Aphis glycines* Matsumura. Thesis, Kansas State University

Diaz-Montano J, Reese JC, Schapaugh WT, Campbell LR (2006) Characterization of antibiosis and antixenosis to the soybean aphid (Hemiptera: Aphididae) in several soybean genotypes. J Econ Entomol 99:1884–1889

Diers BW, Mansur L, Imsande J, Shoemaker RC (1992) Mapping phytophthora resistance loci in soybean with restriction fragment length polymorphism markers. Crop Sci 32:377–383

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relation to the D genome of bread wheat (AABBDD). Genome 34:362–374

Gore MA, Hayes AJ, Jeong SC, Yue YG, Buss GR, Saghai Maroof MA (2002) Mapping tightly linked genes controlling potyvirus infection at the *Rsv*1 and *Rpv*1 region in soybean. Genome 45:592–599

Hartman GL, Domier LL, Wax LM, Helm CG, Onstad DW, Shaw JT, Solter LF, Voegtlin DJ, D'Arcy CJ, Gray ME, Steff ey KL, Isard SA, and Orwick PL (2001) Occurrence and distribution of *Aphis glycines* on soybeans in Illinois in 2000 and its potential control. Plant Health Progress doi: 10.1094/PHP-2001-0205-01-HN.

He FG, Yan FY, Xin WM, Li XP, Wang YQ, Zhang GX (1991) Optimal spraying time and economic threshold of the soybean aphid. Acta Phyt Sinica 18: 155-159.

Hill CB, Crull L, Herman TK, Voegtlin DJ, Hartman GL (2010) A new soybean aphid (Hemiptera: Aphididae) biotype identified. J Econ Entomol 103: 509-515

Hill CB, Kim KS, Crull L, Diers BW, Hartman GL (2009) Inheritance of resistance to the soybean aphid in soybean PI 200538. Crop Sci 49:1193-1200

Hill CB, Li Y, Hartman GL (2004) Resistance to the soybean aphid in soybean germplasm. Crop Sci 44:98–106

Hill CB, Li Y, Hartman GL (2006a) A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. Crop Sci 46:1601–1605

Hill CB, Li Y, Hartman GL (2006b) Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. Crop Sci 46:1606–1608

Kim KS, Hill CB, Hartman GL, Mian MAR, Diers BW (2008) Discovery of soybean aphid biotypes. Crop Sci 48:923–928

Li Y, Hill CB, Carlson SR, Diers BW, Hartman GL (2007) Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group M. Mol Breeding 19:25–34

Mensah C, DiFonzo C, Nelson RL, Wang D (2005) Resistance to soybean aphid in early maturing soybean germplasm. Crop Sci 45:2228–2233

Meng J, Schapaugh WT, Reese J, Smith CM (2010) Inheritance of soybean aphid resistance in soybean genotype K1621. Crop Sci Submitted

Mian MAR, Hammond RB, St Martin SK (2008a) New plant introductions with resistance to the soybean aphid. Crop Sci 48:1055–1061

Mian MAR, Kang ST, Beil SE, and Hammond RB (2008b) Genetic linkage mapping of the soybean aphid resistance gene in PI 243540. Theor Appl Genet 117:955–962.

Mienie CMS, Fourie H, Smit MA, Van Staden J, Botha FC (2002) IdentiWcation of AFLP markers in soybean linked to resistance to *Meloidogyne javanica* and conversion to sequence characterized amplifed regions (SCARs). Plant Growth Reg. 37:157–166

Ostlie K (2002) Managing soybean aphid. University of Minnesota Extension Service, St Paul. http://www.soybeans.umn.edu/crop/insects/aphid/aphid_publication_managingsba.htm. Accessed 16 October 2009

Rector BG, All JN, Parrott WA, Boerma HR (1999) Quantitative trait loci for antixenosis resistance to corn earworm in soybean. Crop Sci 39:531–538

Rector BG, All JN, Parrott WA, Boerma HR (2000) Quantitative trait loci for antibiosis resistance to corn earworm in soybean. Crop Sci 40:233–238

Rice ME, O'Neal M, Pedersen P (2007) Soybean aphids in Iowa-2007. Iowa State Univ. University Extension. http://extension.agron.iastate.edu/soybean/documents/SP247Aphids07.pdf. Accessed 16 October 2009

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM (1998) The nematode resistance gene Mi of tomato confers resistance against the potato aphid. Proc Natl Acad Sci USA 95:9750–9754

Song QJ, Marek LF, Shoemaker RC, Lark KG, Concibido VC, Delannay X, Specht JE, Cregan PB (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122–128

Tamulonis JP, Luzzi BM, Hussey RS, Parrott WA, Boerma HR (1997a) DNA marker analysis of loci conferring resistance to peanut root-knot nematode in soybean. Theor Appl Genet 95:664–670

Tamulonis JP, Luzzi BM, Hussey RS, Parrott WA, Boerma HR (1997b) DNA markers associated with resistance to Javanese root-knot nematode in soybean. Crop Sci 37:783–788

Venette RC, Ragsdale DW (2004) Assessing the invasion by soybean aphid (Homoptera: Aphididae): where will it end? Ann Entomol Soc Am 97: 219D226

Wang S, Basten CJ, Zeng ZB (2008) Windows QTL Cartographer 2.5. Dept of Stat, North Carolina State University, Raleigh. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm.

Wu Z, Schenk-Hamlin D, Zhan W, Ragsdale DW, Heimpel GE (2004) The soybean aphid in China: a historical review. Ann Entomol Soc Am 97:209–218

Yu YG, Saghai Maroof MA, Buss GR, Maughan PJ, Tolin SA (1994) RFLP and microsatellite mapping of a gene for soybean mosaic virus resistance. Phytopathology 84:60–64

Zhang G, Gu C, Wang DC (2009) Molecular mapping of soybean aphid resistance genes in PI567541B. Theor Appl Genet 118:473–482

Zhang G, Gu C, Wang D (2010) A novel locus for soybean aphid resistance. Theor Appl Genet 120: 1183-1191

# CHAPTER 5 - Registration of K1639-2 soybean germplasm resistant to soybean cyst nematode and soybean aphid


## ABSTRACT

Soybean [*Glycine max* (L.) Merr.] germplasm line K1639-2 (Reg. No. GP-365, PI 658491) was developed and released by the Kansas State University Agricultural Experiment Station, Manhattan, KS, as resistance to both soybean aphid (SBA) (*Aphis glycines* Matsumura), and soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe). K1639-2 was developed by crossing R93-174 × 'Northrup King S59-60'. The line was a reselection in the F8 generation from a heterogeneous F4–derived line. K1639-2 has a relative maturity of 5.2, purple fl owers, gray pubescence, yellow seed coats, dark buff hila, tan pod walls, and determinate growth habit. K1639-2 is resistant to SCN HG Type 7 (race 3) and moderately resistant to HG Type 1.2.3.5.6.7 (race 4), and resistant to soybean aphid (Illinois biotype). K1639-2 may be useful for breeders and researchers interested in developing new germplasm with resistance to SBA and SCN.

# INTRODUCTION

The soybean aphid (SBA), *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an introduced pest of soybean in North America. Originally from China and Japan, SBA was first reported in the United States during summer and fall 2000 (Alleman et al., 2002). In August 2002, SBA was first reported in five eastern Kansas counties (Sloderbeck et al., 2003). In fall 2004, 64 Kansas counties reported the presence of this pest (Sloderbeck et al., 2004). In China the SBA has been a threat to soybean productivity (Sun et al., 2000; Wang et al., 1962, 1998; Wu et al., 2004; Yue et al., 1989). Adults and nymphs of *A. glycines* not only extract phloem sap from thehost plant but also can transmit viruses (Guo and Zhang, 1989; Wang et al., 1998). Both direct feeding and virus transmission may cause damage and yield losses. Strategies to minimize damage due to *A. glycines* include chemical, cultural, and biological control and host plant resistance. All these mechanisms have a role in the management of SBA, but host plant resistance represents a potentially important component of integrated pest management. Hill et al. (2004) reported resistance to soybean aphid identified in soybean germplasm. Since the initial report in 2000 of SBA in North America, two distinct biotypes of *A. glycines* have been reported (Kim et al., 2008).

Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is a major pest of soybean in the United States and the world (Wrather et al., 1994; Wrather and Koenning, 2006). In individual fields in Kansas, crop losses of 35 to 40% have been observed (Todd, 1993). Planting soybean varieties with resistance to SCN is an effective method to defend against yield losses due to *H. glycines* (Chen et al., 2001). Although the potential damage of *A. glycines* to soybean in Kansas is still unknown (Sloderbeck et al., 2004), developing lines with resistance to both SBA and SCN may be useful as germplasm or commercial cultivars.

# MATERIALS AND METHODS

K1639-2 (Reg. No. GP-365, PI 658491) is an $F_8$ single-plant selection from K1639. K1639 is an $F_4$–derived line from a cross between R93-174 x 'Northrup King S59-60' made in 1998 at the Agronomy Ashland Research Farm, Manhattan, KS. R93-174 is derived from 'Asgrow A5403' x 'Hutcheson' (Buss et al., 1988). The $F_1$ and $F_3$ generations were grown during the winters of 1998–1999 and 1999–2000, respectively, in Chile. All other generations were grown at the Ashland Research Farm. K1639 was composited in the $F_5$ generation in 2001 at the Ashland Research Farm. In 2002 and 2003, K1639 was evaluated in field trials in Kansas, leading to its evaluation in 2004 in the uniform soybean tests, southern states (Paris, 2004). Screening K1639 for response to SBA (Illinois biotype) was described by Diaz-Montano et al. (2006, 2007a,b). In 2005 eight $F_{8:9}$ progeny of K1639 were evaluated for SBA resistance in an environment-controlled growth chamber under a 25°C day:22°C night temperature regime and a 14:10-h photoperiod. The eight progeny were planted in a completely randomized design with five replicates. When plants reached the V1 developmental stage, each unifoliolate leaf was infested with two adult soybean aphids. A double-sided sticky cage with inner dimensions of 25 by 15 mm was glued on the leaf to restrict aphids' movement and covered with a slightly larger piece of organdy cloth. Eight days after infestation, the SBA adults and nymphs in each cage were counted separately and the ratio of SBA nymphs to adults was calculated. One of the $F_{8:9}$ progeny, K1639-2, was then evaluated for resistance to two populations of the SCN. K1639-2 was planted in soil infested with either HG Type 7 (race 3) or HG Type 1.2.3.5.6.7 (race 4), along with the HG Type indicator lines and 'Lee 74' as the standard susceptible in a completely randomized design with six replicates. Cysts were dislodged from the roots 35 d after planting and counted as described by Niblack et al. (2002). Female indices were calculated on the basis of

the number of cysts recovered from Lee 74 (311 and 213 for HG Types 7 and 1.2.3.5.6.7, respectively).

## CHARACTERISTICS

K1639-2 is a group V maturity (relative maturity 5.2) line with purple flowers, gray pubescence, yellow seed coats, dark buff hila, tan pod walls, and determinate growth habit. In 2002 and 2003, K1639 was evaluated in replicated trials across four Kansas locations. The seed yield of K1639 (2614 kg $ha^{-1}$) was greater than that of 'Manokin' (Kenworthy et al., 1996) (2432 kg $ha^{-1}$; $P < 0.05$). Maturity of K1639 (23 October) was 3 d later than Manokin (20 October; $P < 0.05$). In comparison to '5002T' (Pantalone et al., 2004) in the south uniform regional test in 2004, K1639 averaged 13% less seed yield, 1 d earlier maturity, and 5 cm taller plant height, and had a 0.4 lower lodging score (Table 1 ). Seeds of K1639 averaged 1.5 g 100 $seed^{-1}$ smaller, 13 g $kg^{-1}$ lower protein, and 10 g $kg^{-1}$ lower oil on a 13% moisture basis than seeds of 5002T.

**Table 5-1 Summary of soybean USDA uniform trial, multistate data in 2004.**

| Entry | Yield | Maturity† | Lodging‡ | Plant Height | Seed Weight | Protein§ | Oil |
|---|---|---|---|---|---|---|---|
| No. sites | 7 | 5 | 7 | 7 | 5 | 4 | 4 |
| | kg $ha^{-1}$ | date | 1-5 | cm | g/100 | — g $kg^{-1}$ — | |
| 5002T | 3376 | 1 Oct. | 2.0 | 66 | 14.3 | 401 | 201 |
| K1639 | 2937 | 30 Sept. | 1.6 | 71 | 12.8 | 388 | 191 |
| $LSD_{0.05}$ | 376 | 1 | 0.5 | 6.6 | 1.1 | 13 | 8 |

†Date when 95% of the pods have ripened, as indicated by their mature pod color.
‡Visual score: 1 = almost all plants erect, 5 = almost all plants down.
§Protein and oil reported on a 130 g $kg^{-1}$ moisture basis.

In screening for response to SBA, K1639 showed a level of resistance similar to the resistance exhibited by 'Jackson' and 'Dowling' (Craigmiles et al., 1978; Diaz-Montano et al., 2006, 2007a,b; Johnson, 1958). K1639 has the same SBA resistant ancestor, 'CNS', as Dowling. However, K1639 appeared to be heterogeneous for resistance to SBA. Evaluation of the eight $F_{8:9}$ progeny of K1639 revealed that seven of the eight lines were phenotypically similar to each other and the K1639 bulk, in visual plant traits, and resistant to SBA. The remaining line was susceptible to SBA. Aphid reproduction on the seven resistant lines ranged from 4.4 to 8.4, whereas the susceptible line had 35 total adults and nymphs ($LSD_{0.05}$ = 8.8). Aphid reproduction among the resistant lines was not significantly different, but K1639-2 was selected for increase and release because of seed supply. The aphid number on K1639-2 was 2.2 adults per plant and 3.6 nymphs per plant, both of which were significantly ($P < 0.0001$) less than those on susceptible check KS4202 (13 adults per plant and 33 nymphs per plant).

Screening K1639-2 for resistance to SCN showed a similar reaction to SCN HG Types (races) as PI 88788 (Tables 2 and 3 ). K1639-2 was resistant to SCN HG Type 7 (race 3) and moderately resistant to HG Type 1.2.3.5.6.7 (race 4) in greenhouse tests at Manhattan, KS, in 2007 and 2008. Average SCN female indexes on K1639-2 for HG Types 7 and 1.2.3.5.6.7 were 1.4 and 14.2, respectively, based on a SCN female index of 100 for the susceptible check (Niblack et al., 2002). In the same tests, female indexes for PI 88788 for each HG Type averaged 2.7 and 13.0, respectively.

**Table 5-5-2 HG Type determination and response of K1639-2 to a "race 3" population of *Heterodera glycines*.**

| Line | No. plants | FI† | Std. err. |
|---|---|---|---|
| PI 548402 | 7 | 2.37 | 2.23 |
| PI 88788 | 6 | 2.70 | 0.71 |
| PI 90763 | 6 | 0.07 | 0.07 |
| PI 437654 | 6 | 0.00 | 0.00 |
| PI 209332 | 7 | 1.40 | 0.37 |
| PI 89772 | 6 | 0.00 | 0.00 |
| PI 548316 | 5 | 40.43 | 8.10 |
| K1639-2 | 8 | 1.40 | 0.31 |

† Female Index (FI) = (number of females on indicator line/average number of females on Lee74) × 100; number of females on Lee74 averaged 311.

**Table 5-3 HG Type determination and response of K1639-2 to a "race 4" population of *Heterodera glycines*.**

| Line | No. plants | FI† | Std. err. |
|---|---|---|---|
| PI 548402 | 6 | 19.33 | 4.21 |
| PI 88788 | 5 | 12.96 | 1.72 |
| PI 90763 | 5 | 12.58 | 2.37 |
| PI 437654 | 4 | 9.74 | 9.74 |
| PI 209332 | 6 | 10.64 | 2.81 |
| PI 89772 | 5 | 16.71 | 3.58 |
| PI 548316 | 6 | 15.41 | 4.26 |
| K1639-2 | 5 | 14.18 | 2.64 |

† Female Index (FI) = (number of females on indicator line/average number of females on Lee74) × 100; number of females on Lee74 averaged 213.

## AVAILABILITY

Seed of K1639-2 was deposited in the USDA Soybean Germplasm Collection. Packets of 50 seeds of K1639-2 may be obtained on written request. Appropriate recognition is requested when this germplasm contributes to the development of a new cultivar or germplasm. Requests for seed should be directed to the corresponding author.

# References


Alleman, R.J., C.R. Grau, and D.B. Hogg. 2002. Soybean aphid host range and virus transmission efficiency. Proc. Wisc. Fertilizer Agline Pest Management Conf. (http://www.soils.wisc.edu/extension/FAPM/fertaglime02.htm).

Buss, G.R., H.M. Camper, Jr., and C.W. Roane. 1988. Registration of 'Hutcheson' soybean. Crop Sci. 28:1024.

Chen, S.Y., P.M. Porter, J.H. Orf, C.D. Reese, W C. Stienstra, N.D. Young, D.D. Walgenbach, P.J. Schaus, T.J. Arlt, F.R. Breitenbachand, and M.G. Mitchum. 2001. Soybean cyst nematode population development and associated soybean yields of resistant and susceptible cultivars in Minnesota. Plant Dis. 85(7):760-766.

Craigmiles, J.P., E.E. Hartwig, and J.W. Sij. 1978. Registration of Dowling soybean. Crop Sci 18(6):1094.

Diaz-Montano, J., J.C. Reese, W.T. Schapaugh, and L.R. Campbell. 2006. Characterization of antibiosis and antixenosis to the soybean aphid (Hemiptera:Aphididae) in several soybean genotypes. J. Econ. Ento. 99:1884-1889.

Diaz-Montano, J., J.C. Reese, W.T. Schapaugh, and L.R. Campbell. 2007a. Chlorophyll loss caused by soybean aphid (Hemiptera: Aphididae) feeding on soybean. J. Econ. Ento. 100:1657-1662.

Diaz-Montano, J., J.C. Reese, J. Louis, L.R. Campbell, and W.T. Schapaugh. 2007b. Feeding behavior by the soybean aphid (Hemiptera:Aphididae) on resistant and susceptible soybean genotypes. J. Econ. Ento. 100:984-989.

Guo, J.Q., and M.H. Zhang. 1989. Study on the important vectors of soybean mosaic virus and their transmission efficiency. Soybean Sci. 8:55-63.

Hill, C.B., Y. Li, and G.L. Hartman. 2004. Resistance to the soybean aphid in soybean germplasm. Crop Sci. 44:98-106.

Johnson, H.W. 1958. Registration of soybean varieties. VI. Agron. J. 50(11): 690.

Kenworthy, W.J., J.G. Kantzes, L.R. Krusberg, and S. Sardanelli. 1996. Registration of 'Manokin' soybean**.** Crop Sci. 1996 36: 1079.

Kim, Ki-Seung, C.B. Hill, G.L. Hartman, M.A. Rouf Mian, and B.W. Diers. 2008. Discovery of soybean aphid biotypes. Crop Sci. 48:923-928.

Niblack, T.L., P.R. Arelli, G.R. Noel, C.H. Opperman, J.H. Orf, D.P. Schmitt, J.G. Shannon, and G.L. Tylka. 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines.* J. Nematol. 34:279–288.

Pantalone, V.R., F.L. Allen, and D. Landau-Ellis. 2004. Registration of '5002T' soybean. Crop Sci. 44:1483.

Paris, R.L. (ed.). 2004. Uniform soybean tests southern states: 2004. USDA-ARS, Stoneville, MS.

Sloderbeck, P. E., J. C. Reese, R. J. Whitworth, C. M. Smith, R. A. Higgins, W. T. Schapaugh, R. E. Wolf and Doug J. Jardine. 2003. The Soybean Aphid. Kansas State University Agricultural Experiment Station and Cooperative Extension Service. MF-2582. http://www.oznet.ksu.edu/library/entml2/mf2582.pdf.

Sloderbeck, P.E., R.J. Whitworth, and R.A. Higgins. 2004. Soybean insect management 2004. Kansas State University Agricultural Experiment Station and Cooperative Extension Service. MF-743.

Soybean Aphid Watch. 2003. Facts. http://www.pmcenters.org/Northcentral/Saphid/Facts.htm.

Sun, B., S.B. Liang, and W.X. Zhao. 2000. Outbreak of the soybean aphid in Suihua prefecture in 1998 and its control methods. Soybean Bull. 1:5.

Todd, T., 1993. Soybean planting date and maturity planting effects on *Heterodera glycines* and *Macrophomina phaseolina* in Southeastern Kansas. Supp. J. Nematol. 25(45):731-737.

Wang, C.R., J.G. Chen, Y.R. Guo, X.Y. Gong, Z.F. Xu, and C. Lin. 1998. Development and control of soybean apid, Aphis glycines, in Heilongjiang province. Soybean Bull. 6:15.

Wang, C.L., N.J. Siang, G.S. Chang, and H.F. Chu. 1962. Studies on the soybean aphid, *Aphis glycines* Mastumura. Acta Entomol. Sinica. 11:31-44.

Wrather, J.A., T.R. Anderson, D.M. Arsyad, J. Gai, L.D. Ploper, A. Porta-Puglia, H.H. Ram, J.T. Yorinori. 1994. Soybean disease loss estimates for the top 10 soybean producing countries in 1994. Plant Dis. 81:107-110.

Wrather, J.A., and S.R. Koenning. 2006. Estimates of disease effects on soybean yields in the United States 2003 to 2005. J. Nematol. 38:173-180.

Wu, Z., D. Schenk-Hamlin, W. Zhan, D.W. Ragsdale, and G.E. Heimpel. 2004. The soybean aphid in China: a historical review. Ann. Entomol. Soc. Am. 97:209-218.

Yue, D.R., S.G. Guo, and Y.L. Shan. 1989. Resistance of wild soybean *Glycine soja* to *Aphis glycines*. I. Screening for resistant varieties. Jilin Agric. Sci. 3:15-19.

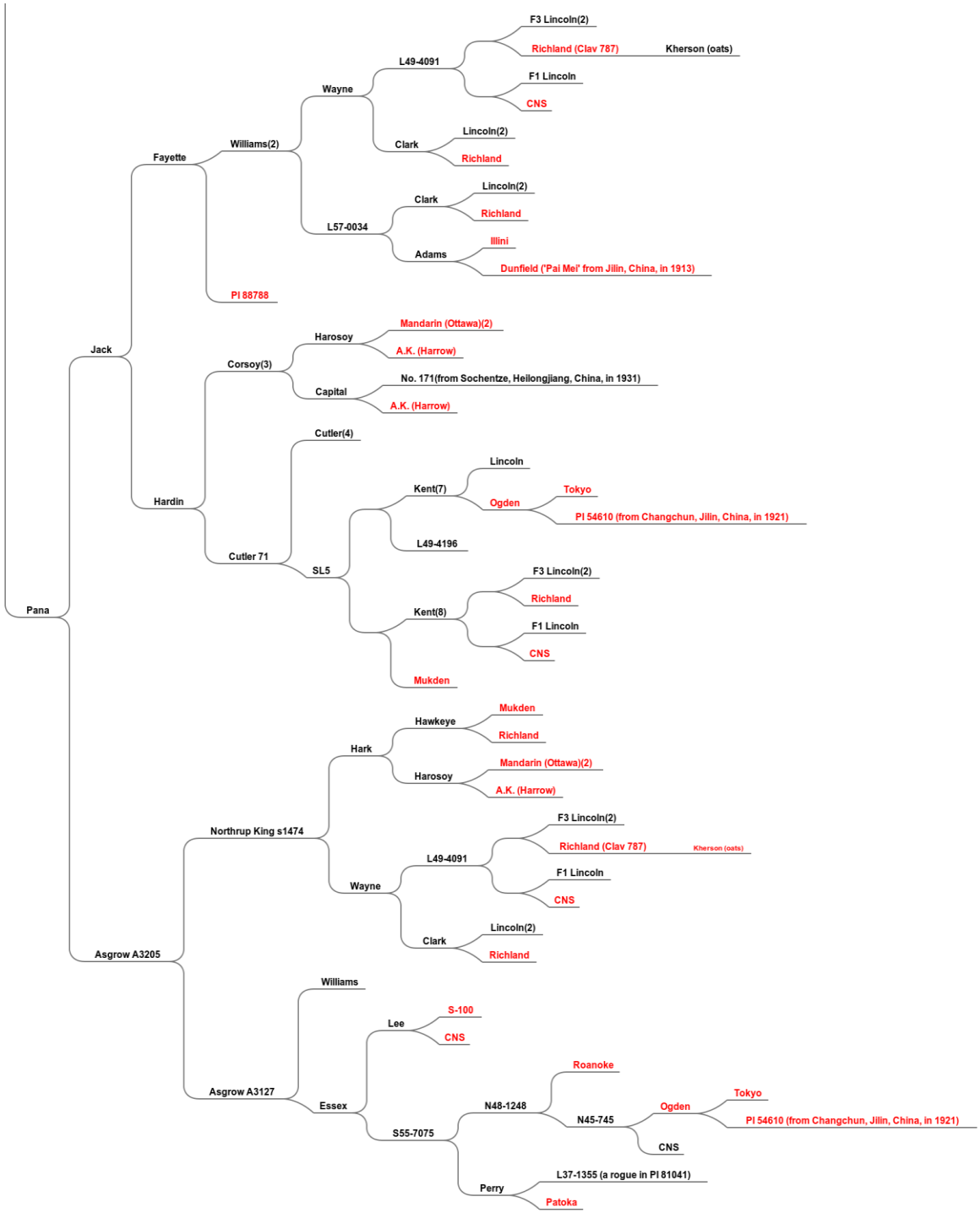
# Appendix A - Analysis of resistance gene in K1639

**Table A-1 Phenotypic segregation of $F_2$ generation from crosses of K1639-2/Jackson and K1639-2/Dowling.**

| | ADULTS | | | | NYMPHS | | | | TOTAL | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ENTRY | MEAN | ± | SE | t group | MEAN | ± | SE | t group | MEAN | ± | SE | t group | PEDIGREE |
| JACKSON | 0.1 | ± | 0.3 | ab† | 0.0 | ± | 0.5 | a | 0.1 | ± | 0.8 | a | |
| SBA 06-1531 | 0.0 | ± | 0.3 | a | 0.3 | ± | 0.7 | ab | 0.3 | ± | 0.9 | a | K1639-2/JACKSON |
| SBA 06-1555 | 0.1 | ± | 0.3 | ab | 0.2 | ± | 0.6 | ab | 0.3 | ± | 0.8 | a | K1639-2/JACKSON |
| SBA 06-1532 | 0.1 | ± | 0.3 | ab | 0.4 | ± | 0.6 | ab | 0.5 | ± | 0.8 | ab | K1639-2/JACKSON |
| SBA 06-1543 | 0.4 | ± | 0.3 | abc | 0.3 | ± | 0.6 | ab | 0.7 | ± | 0.8 | ab | K1639-2/JACKSON |
| SBA 06-1545 | 0.6 | ± | 0.3 | abc | 0.4 | ± | 0.6 | ab | 1.0 | ± | 0.9 | ab | K1639-2/JACKSON |
| SBA 06-1539 | 0.3 | ± | 0.3 | abc | 0.9 | ± | 0.6 | ab | 1.2 | ± | 0.8 | ab | K1639-2/JACKSON |
| SBA 06-1533 | 0.7 | ± | 0.3 | abc | 0.7 | ± | 0.6 | ab | 1.4 | ± | 0.8 | ab | K1639-2/JACKSON |
| SBA 06-1537 | 0.9 | ± | 0.3 | bc | 0.8 | ± | 0.6 | ab | 1.7 | ± | 0.9 | ab | K1639-2/JACKSON |
| SBA 06-1557 | 0.5 | ± | 0.3 | abc | 1.8 | ± | 0.6 | b | 2.3 | ± | 0.8 | ab | K1639-2/JACKSON |
| SBA 06-1551 | 1.0 | ± | 0.3 | c | 1.6 | ± | 0.6 | ab | 2.6 | ± | 0.8 | b | K1639-2/JACKSON |
| SBA 06-1763 | 0.2 | ± | 0.3 | abc | 0.1 | ± | 0.6 | a | 0.3 | ± | 0.8 | ab | K1639-2/DOWLING |
| SBA 06-1755 | 0.2 | ± | 0.3 | abc | 0.2 | ± | 0.6 | ab | 0.4 | ± | 0.8 | ab | K1639-2/DOWLING |
| SBA 06-1744 | 0.3 | ± | 0.3 | abc | 0.1 | ± | 0.6 | a | 0.4 | ± | 0.9 | ab | K1639-2/DOWLING |
| SBA 06-1756 | 0.2 | ± | 0.3 | abc | 0.3 | ± | 0.6 | ab | 0.6 | ± | 0.9 | ab | K1639-2/DOWLING |
| SBA 06-1757 | 0.3 | ± | 0.3 | abc | 0.3 | ± | 0.6 | ab | 0.6 | ± | 0.8 | ab | K1639-2/DOWLING |
| SBA 06-1753 | 0.4 | ± | 0.3 | abc | 0.4 | ± | 0.6 | ab | 0.8 | ± | 0.8 | ab | K1639-2/DOWLING |
| SBA 06-1751 | 0.4 | ± | 0.3 | abc | 0.4 | ± | 0.6 | ab | 0.8 | ± | 0.8 | ab | K1639-2/DOWLING |
| SBA 06-1748 | 0.6 | ± | 0.3 | abc | 0.6 | ± | 0.6 | ab | 1.1 | ± | 0.9 | ab | K1639-2/DOWLING |
| SBA 06-1747 | 0.8 | ± | 0.3 | abc | 1.0 | ± | 0.6 | ab | 1.8 | ± | 0.9 | ab | K1639-2/DOWLING |
| SBA 06-1737 | 0.9 | ± | 0.3 | bc | 1.1 | ± | 0.6 | ab | 2.0 | ± | 0.8 | ab | K1639-2/DOWLING |
| KS4202 | 5.5 | ± | 0.3 | d | 12.7 | ± | 0.6 | c | 18.2 | ± | 0.8 | c | |

† Mean aphid numbers followed by different letters are significantly different (P<0.05; t-test).

# Appendix B - Pedigree of K1621


Pana
Jack
Fayette
Williams(2)
Wayne
L49-4091
F3 Lincoln(2)
Richland (Clav 787)
Kherson (oats)
F1 Lincoln
CNS
Clark
Lincoln(2)
Richland
L57-0034
Clark
Lincoln(2)
Richland
Adams
Illini
Dunfield ('Pai Mei' from Jilin, China, in 1913)
PI 88788
Hardin
Corsoy(3)
Harosoy
Mandarin (Ottawa)(2)
A.K. (Harrow)
Capital
No. 171(from Sochentze, Heilongjiang, China, in 1931)
A.K. (Harrow)
Cutler 71
Cutler(4)
SL5
Kent(7)
Lincoln
Ogden
Tokyo
PI 54610 (from Changchun, Jilin, China, in 1921)
L49-4196
Kent(8)
F3 Lincoln(2)
Richland
F1 Lincoln
CNS
Mukden
Asgrow A3205
Northrup King s1474
Hark
Hawkeye
Mukden
Richland
Harosoy
Mandarin (Ottawa)(2)
A.K. (Harrow)
Wayne
L49-4091
F3 Lincoln(2)
Richland (Clav 787)
Kherson (oats)
F1 Lincoln
CNS
Clark
Lincoln(2)
Richland
Asgrow A3127
Williams
Lee
S-100
CNS
Essex
S55-7075
N48-1248
Roanoke
N45-745
Ogden
Tokyo
PI 54610 (from Changchun, Jilin, China, in 1921)
CNS
Perry
L37-1355 (a rogue in PI 81041)
Patoka