# QTL Mapping of soybean aphid resistance gene in soybean genotype K1621

J. Meng[1], W.T. Schapaugh, Jr.[1], J.C. Reese[2], C.M. Smith[2]
[1]Department of Agronomy [2] Department of Entomology, Kansas State University

## Introduction

- The soybean aphid (*Aphis glycines* Matsumura) has become one of the most serious threats to soybean production in North America since 2000.
- Soybean aphids causes damage by direct feeding and transmitting virus diseases.
- Plant resistance is an efficient, economical, and environmentally friendly method to control soybean aphid.
- Three resistance genes, *Rag1, 2,* and *3* have been mapped to chromosomes 7, 13, and 16, respectively.
- Soybean genotype K1621 has moderate resistance to soybean aphid biotype 1 that differs from the resistance provided by *Rag1*.

## Objectives

- To map and validate the soybean aphid resistance gene in K1621 using simple sequence repeat (SSR) markers
- To investigate the ancestors of the resistance in K1621

## Materials and methods

**Population development:**
Mapping population: 150 $F_{2:3}$ families from a cross of KS4202 (susceptible) and K1621 (moderate resistant). Each $F_{2:3}$ family contained up to 22 $F_3$ plants.
Validation population: 106 $F_{2:4}$ families from cross of K1621 and Dowling (*Rag1*). Each family contained up to 14 $F_4$ plants.

**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. Plants were grown individually in plastic Cone-tainers and arranged in a randomized complete block design with three (mapping population) or two (validation population) replications.
Each plant was infested at the V1 stage (Fehr and Caviness, 1977) with 2 adult aphids on each unifoliolate leaf. Soybean aphid resistance was evaluated by counting the total number of aphids on each plant 7 days following infestation.

**Genotyping:**
Non-expanded trifoliolate leaves from each line were bulk harvested for use in isolating genomic DNA using the CTAB (hexadecyltrimethyl ammonium bromide) method. A total of 543 SSR markers covering the soybean genome (Song et al. 2004) were screened for polymorphism between parents. Polymorphic markers were used to genotype the mapping population with an ABI 3730 sequencer. Amplification results were visualized and scored with GeneMarker 1.7.

**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. Single marker analysis (SMA) and composite interval mapping (CIM) were performed using QTLCartorgrapher2.5 with the standard model Zmapqtl6.

**Ancestry study:**
Markers tightly linked to soybean aphid resistance in both populations were used to genotype 19 ancestors of K1621, KS4202, K1621, and Dowling. 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). Broad sense heritability was 60%.

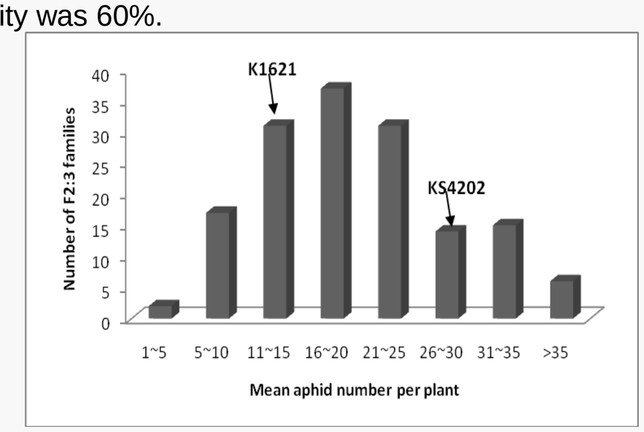


**Fig.1** Frequency distribution of soybean aphid number per plant for 150 $F_{2:3}$ families derived from the cross of KS4202 and K1621. Arrows indicate parents.

**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. 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.

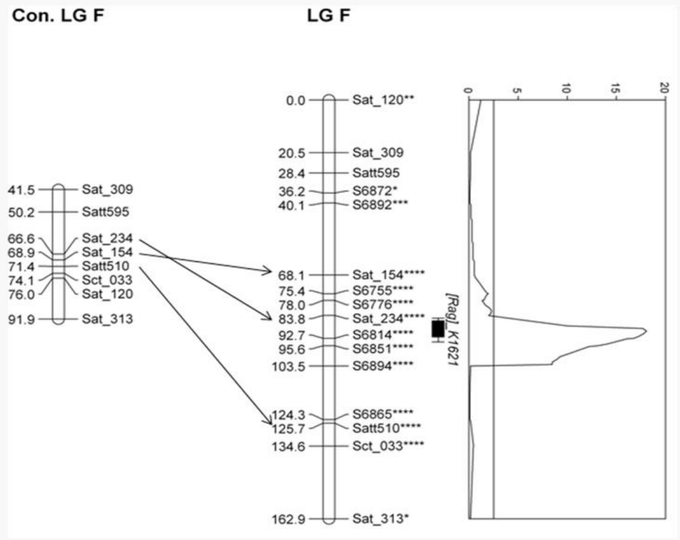


**Fig. 2** Corresponding segments of consensus soybean chromosome 13 (Con. LG F) and chromosome 13 (LG F) constructed in this study. The location of the soybean aphid resistance QTL was identified using the composite interval mapping method. 1-LOD and 2-LOD support intervals of QTL are marked by thick 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 (Fig. 3). Single marker analysis confirmed that four markers, including marker S6814, are closely linked to the QTL on chromosome 13 (LG F; Table 1).

Marker 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).

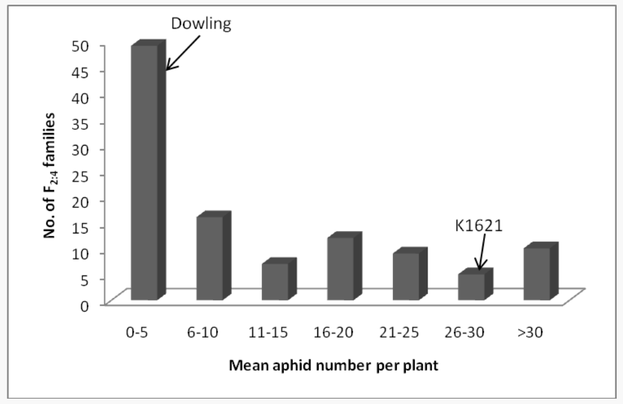


**Fig.3** Frequency distribution of soybean aphid number per plant for 106 $F_{2:4}$ families derived from the cross of K1621 and Dowling. Arrows indicate parents.

**Table 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). 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.

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

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