

Mapping and Characterization of a New Soybean Aphid Resistance Gene in PI567301B

Rouf Mian, Tae-Hwan Jun and Andrew Michel



Update on Soybean Aphid resistance Research in Ohio

- Conventional soybean germplasm with **antibiosis** resistance to aphid biotypes 1 and 2 (Rag2 from PI243540)
- Conventional soybean germplasm with **antixenosis** resistance to aphid biotype 1 and 2 (Rag?? from PI567301B)
- Pyramiding Rag1 and Rag2 genes in MG II and III breeding lines
- Mapping of aphid resistance genes in PI567301B and PI567324
- Fine mapping of Rag2 in PI243540 (<50 kb)
- NILs: BC6 for Rag2 (PI243540) and Rag? (PI567301B)
- Metabolomics, transcriptomics, and proteomics studies using the BC5/BC6 NILs
- Screening of more PIs for new sources of soybean aphid resistance
- Development of SA SSR markers and SA genetic diversity studies
- Several projects are under way on the soybean aphid in Dr. Andy Michel's lab



Wyandot Rag2 Pmd

- Wyandot Rag2 Rmd is a late MG-II (2.9) conventional soybean germplasm with antibiosis resistance to soybean aphid biotype 1 and 2 and powdery mildew
- Single dominant gene *Rag2* from PI 243540 (Mian et al. 2008)
- Single dominant gene *Pmd* PI243540 (Kang and Mian 2010)
- No yield drag and often yields higher than Wyandot
- Markers for melting curve analyses on LightCycler 480 for both genes, closely linked SSR markers also available
- **Susceptible to biotype 3 of soybean aphid**



Mapping Antixenosis Resistance in PI 567301B

- 203 F7-derived RILs from Wyandot x PI 567301B cross
- Phenotyping in greenhouse by scoring at 14 and 28 DAI
- Phenotyping in field cages by scoring at 14 and 28 DAI
- Genotyping with SNP and SSR markers



Resistant parent: PI 567301B



Susceptible parent: Wyandot

Mian et al. 2008. New plant introductions with resistance to the soybean aphid.
Crop Sci. 48: 1055-1061

Phenotyping



Field test (4 seedlings/RIL)



Greenhouse test (4 seedlings/RIL)

Phenotypic scores assigned at 14 and 28 DAI using a 1-5 scale based on number of aphids and plant health according to Mian et al. (2008): Crop Sci. 48: 1055-1061

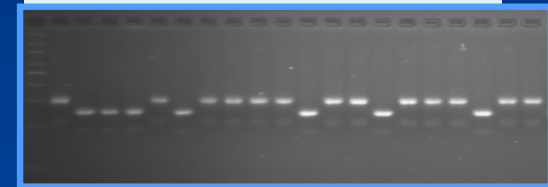


Molecular marker screening

- ◇ SNP Genotyping: Golden gate assay of 94 RILs with 1536 SNPs Panel (Courtesy of DRs. Hyten/Cregan, USDA-ARS)
 - ☞ 607 Polymorphic SNPs
 - ☞ 516 SNP markers used in genetic mapping
- ◇ The QTL regions (chromosome 8 and 13) detected by single factor ANOVA were saturated using SSR markers



SNPs: Illumina BeadStation
500G

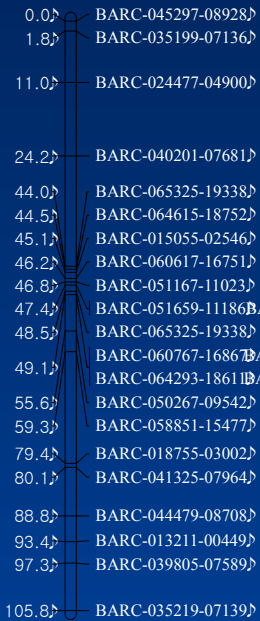


SSRs: 4% high resolution agarose gel

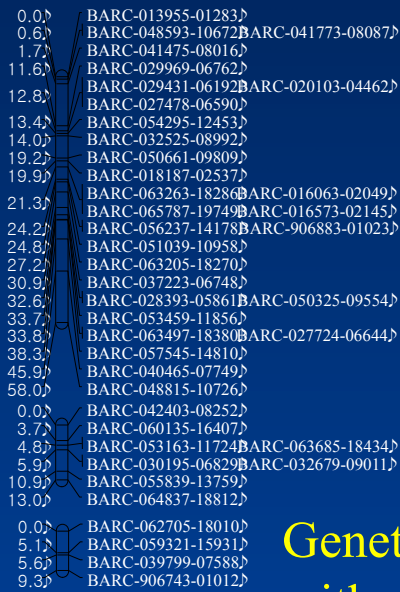
Genetic mapping and QTL Identification

- ◇ Genetic map was constructed using JoinMap 4.0 program
- ◇ Association between markers and phenotypes tested by single factor ANOVA
- ◇ QTL interval mapping using MapQTL 5.0 program
- ◇

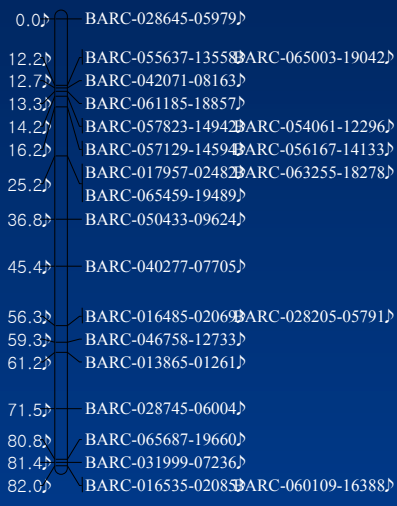
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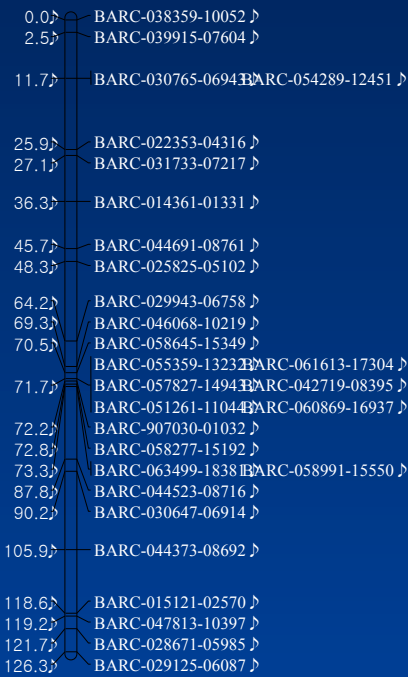
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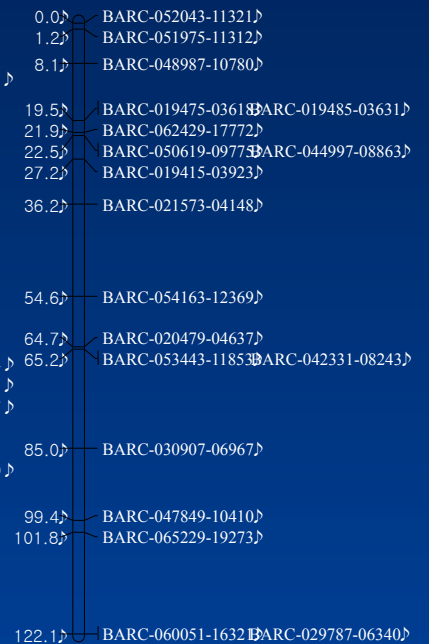
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Chr. 4

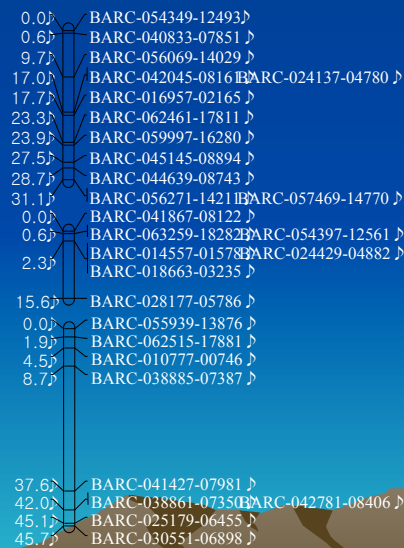


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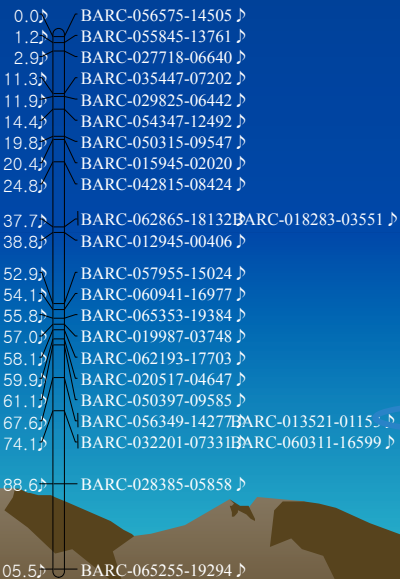


Genetic map: 2200 cM with some large gaps Based on 94 RILs

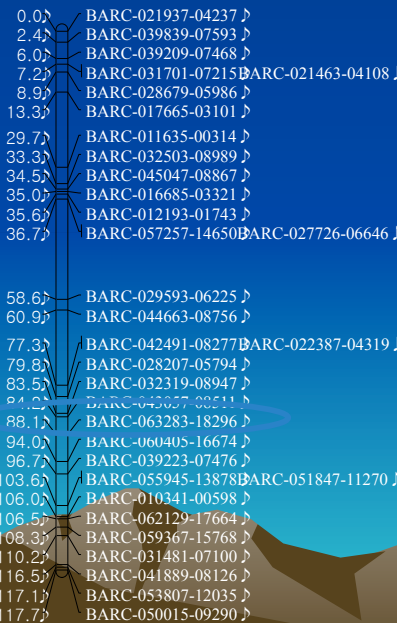
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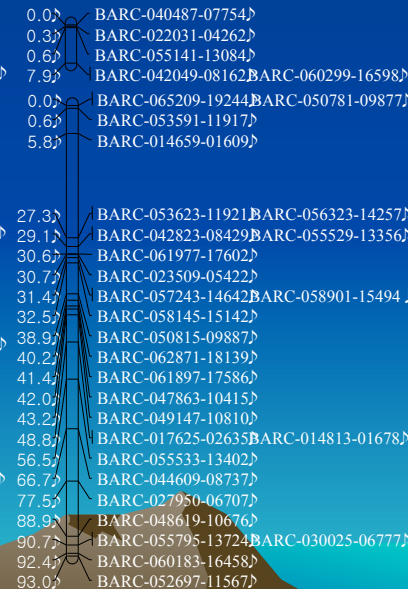
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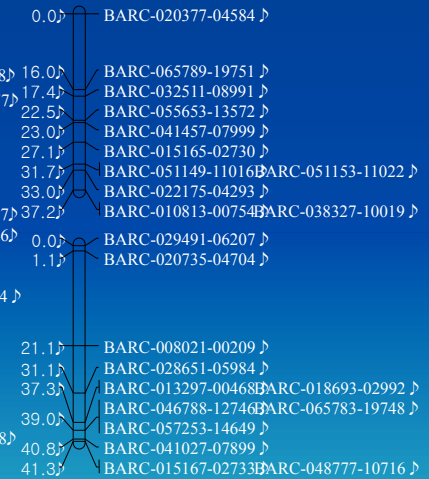
Chr. 8



Chr. 9



Chr. 10



Chr. 11

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Chr. 13

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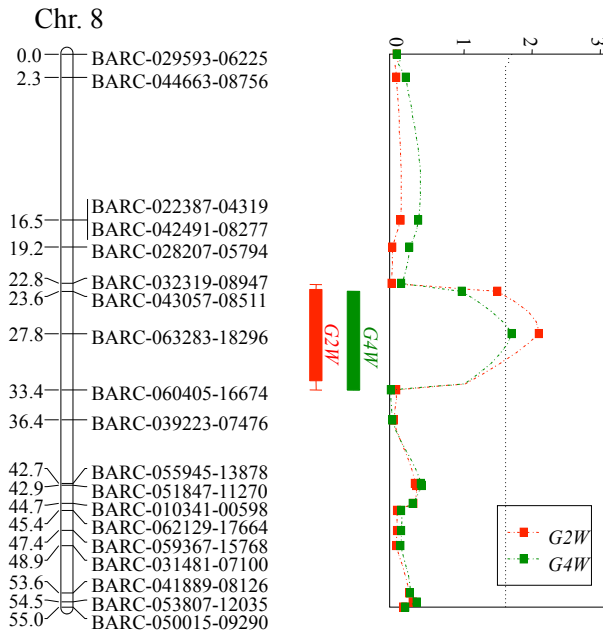
QTL identification by single factor ANOVA

Trial	Chromosome	Position (cM)	Marker	R^2	P value
GH2W	8	94.1	BARC-063283-18296	9.8	0.0027
	13	55.8	BARC-060107-16382	86.2	< 0.0001
GH4W	8	94.1	BARC-063283-18296	8.0	0.0068
	13	55.8	BARC-060107-16382	91.0	< 0.0001
Field2W	8	94.1	BARC-063283-18296	6.7	0.014
	13	55.8	BARC-060107-16382	83.6	< 0.0001
Field4W	8	94.1	BARC-063283-18296	6.3	0.017
	13	55.8	BARC-060107-16382	85.5	< 0.0001

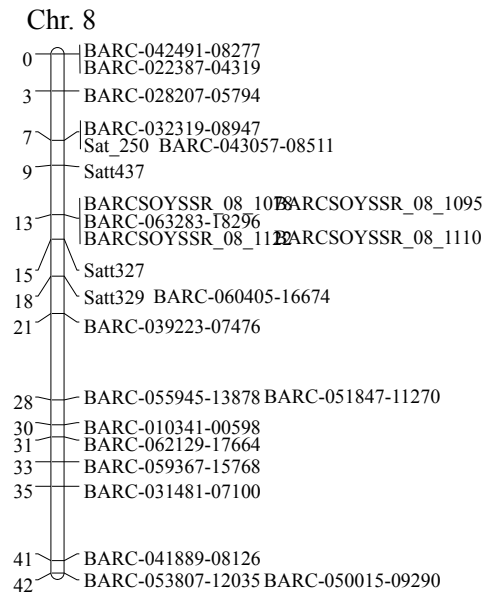
QTL identification by composite interval mapping

Trial	Chrom	Flanking markers	LOD	R^2	a
203 RIL lines					
GH2W	8	BARCSOYSSR_08_1095 - BARCSOYSSR_08_1110	2.0	4.6	-0.31
	13	BARCSOYSSR_13_1134	78.2	84.1	1.31
GH4W	8	BARCSOYSSR_08_1110	1.1	2.4	-0.28
	13	BARCSOYSSR_13_1134	107.2	91.5	1.71
Field2W	13	BARCSOYSSR_13_1134	56.7	74.9	1.42
Field4W	13	BARCSOYSSR_13_1134	65.7	79.3	1.46

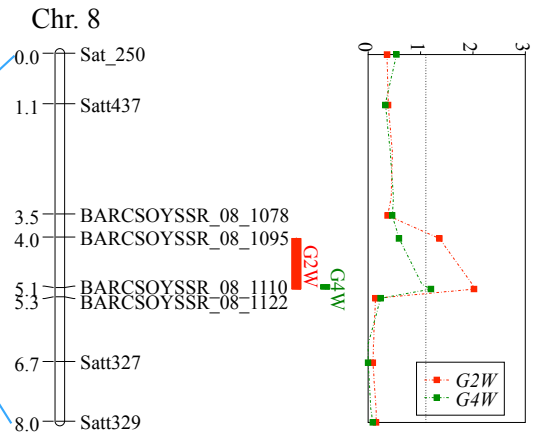
94 RILs



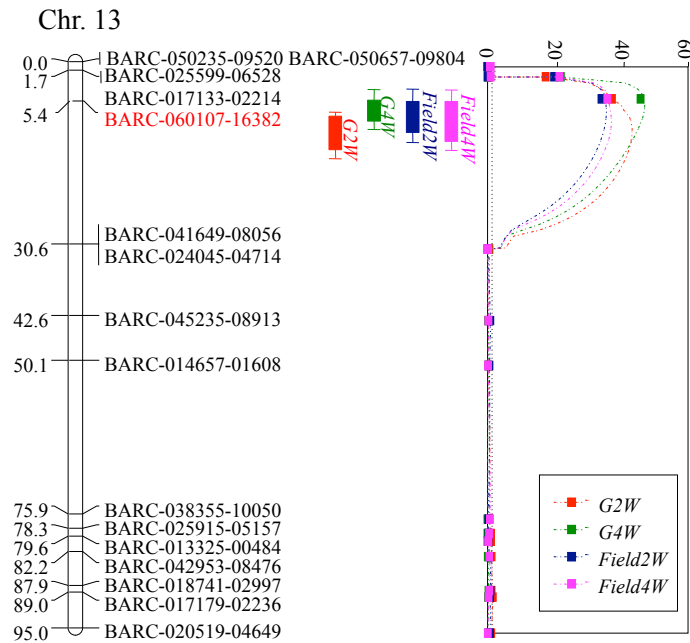
94 RILs



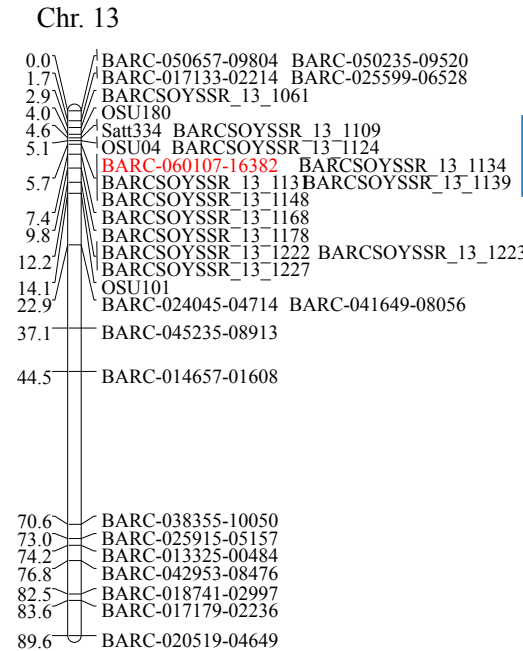
203 RILs



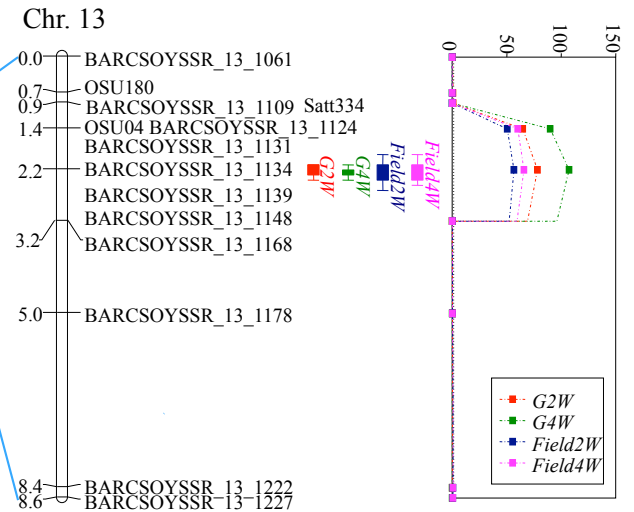
94 RILs



94 RILs



203 RILs



Is the major gene on Chromosome 13 Rag2??

Predicted location of the gene is between 29,036,526 – 29,548,838; ~500 kb interval

Rag2 gene in PI 200538 located between 29,212,318 – 29,266,469; ~50kb interval
(Kim et al. 2010. TAG:121:599–610)



Line name	No. of aphids 5 DAI
PI243540 (Rag2)	0.3
PI567301B (Rag?)	20.0
PI567324	22.0
Wyandot	50.0
LSD _{0.05}	9.0

No Choice Test: caged 4 adult aphids on a leaflet for five days (4-replicates)

Is the major gene on Chrome 13 Rag2??



PI243540 (Rag2)



PI567301B (Rag?)



Wyandot -susceptible

5 adult biotype 2 aphids for 5 days

Detached leaf assays with these and other lines have been conducted dozens of times in Michel and Mian labs for many times over last three years and every time these differences were found

Summary

- Mapped a major gene with **antixenosis** resistance to soybean aphid biotype 1 and 2 on chr 13 in PI567301B
- This gene mapped close to **antibiosis** resistance gene Rag2
- The gene in PI567301B is not Rag2 unless the same gene is acting differently in different soybean background
- We now have BC6 NILs of the two genes in the same background (Wyandot) to resolve this issue
- Fine mapping of the gene



Acknowledgements

- Collaborators: OSU - Drs. Michel, Hammond, Phelan, Mittapalli, McHale, Graham; Others: Stacy & Nguyen (UM Columbia), Kang (Korea), Li (China)
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- Members of my lab group
- USDA-ARS, USB, NCSRP, OSC and OARDC/OSU for financial and material supports

