**Development of Aphid Resistant Germplasm Through a Combination of Germplasm and Genomic** Resources **Brian Diers, Ki-Seung Kim,** Jianping Wang, Carolyn Bonin, **Curt Hill, Glen Hartman, Matt** Hudson

# Outline

- Background on aphid resistance.
- Mapping of aphid resistance genes (*Rag1* and *Rag2*).
- Efforts in cloning *Rag1* and *Rag2*.
- Screening germplasm to identify new aphid resistance genes.
- SNP haplotypes near *Rag2*.

## **Soybean Aphid**

- Soybean aphids (*Aphis glycines* Matsumura) are native to Asia and were first identified as a soybean pest in North America in 2000.
- Spread throughout the northern US soybean production region and southern Canada.
- Estimated .1 to 2.5 million hectares sprayed annually to control the pest.



#### Antibiosis Resistance in Dowling, Jackson and PI 200538

#### **Aphid Mortality**



Li, Y., C.B. Hill, and G.L. Hartman. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera : Aphididae). Journal of Economic Entomology 97: 1106-1111.

#### Antibiosis Resistance in Dowling, Jackson and PI 200538

**Aphid Mortality** 

**Aphid fecundity** 



Li, Y., C.B. Hill, and G.L. Hartman. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera : Aphididae). Journal of Economic Entomology 97: 1106-1111.

#### Resistance in Dowling and PI 200538 Mapped

- Resistance genes were mapped in populations developed by crossing resistant and susceptible plants.
- The populations were evaluated for resistance in greenhouse tests.



#### Map Location of the Aphid Resistance Genes Rag1 and Rag2

Rag1 from Dowling Chromosome 7 (LG M)



Rag2 from PI 200538 Chromosome 13 (LG F)



## **Breeding for Aphid Resistance**

- MAS has been successfully used to develop cultivars with *Rag1* and *Rag2*.
- Aphid resistant cultivars have been released to growers.

#### **Resistant Line**





#### Susceptible Line



#### Fine Mapping Rag1 and Rag2

- Fine map *Rag1* and *Rag2* to provide better markers for MAS and to build resources for cloning.
- Identified recombinant plants from populations with markers flanking the genes.
- Tested 1,824 plants for *Rag1* and 5,783 plant for *Rag2*.



#### Fine Mapping Rag1

Physical position (Mb)	5.47	5.48	5.49	5.55	5.58	5.61	5.64						
	83A	25A	21A	56B	65906.2	46169.7	27A		Apł	nid numl	per	_	
Plant							$\rightarrow$	Pheno	R	н	S	P > F	R <sup>2</sup>
100	R	R	R	н	н	н	н	Seg	71	62	657	<0.0001	0.97

# Recombinant plant



Progeny

#### **Rag1** Fine Mapping

Physical position (Mb)	5.47	5.48	5.49	5.55	5.58	5.61	5.64						
	83A	25A	21A	56B	65906.2	46169.7	27A		Apł	nid numb	er		
Plant								Pheno	R	Н	S	P > F	R <sup>2</sup>
4	R	R	R	R	R	R	Н	Res	62	47	57	0.2043	0.08
82	н	н	н	R	R	R	R	Res	49	58	55	0.2071	0.08
100	R	R	R	Н	н	н	н	Seg	71	62	657	<0.0001	0.97
6	н	н	Н	н	н	н	Н	Seg	47	60	628	<0.0001	0.91
48	NT	NT	NT	NT	S	S	NT	Sus	941	953	984	0.2080	0.17
К39	S	S	S	S	S	н	н	Sus	774	761	770	0.9010	0.01

ر *Rag1* 115 kb interval

#### Rag 1 and Rag2 Fine Mapping

- *Rag1* mapped to a 115 kb interval.
  - Interval contains 14 genes in Williams 82 with predicted expression. Two are NBS-LRR genes.



#### Rag 1 and Rag2 Fine Mapping

• *Rag2* mapped to a 54 kb interval.

 Interval contains 7 genes in Williams 82 with predicted expression. One is a NBS-LRR.

- Are these genes present in the resistance sources? (Williams 82 susceptible)
- Need to clone these regions from resistant genotypes.
- Collaborating with a molecular biologist (Matt Hudson) in cloning.

#### **Dowling Fosmid Library Screened**



Selected clones were sequenced on a 454 resulting in a single contig.

#### Comparison of Williams 82 and Dowling in *Rag1* Region



The two NBS-LRR genes from Dowling are being transformed into soybean.

## Next Step in Aphid Resistance Research

- Are there new genes in the germplasm collection?
- Over 40 new sources of aphid resistance have been identified from the germplasm collection by Hartman's group.
- Do these have new resistance genes?





#### **Genetic Tests of Resistance Sources**

	Significant Rag	Rag region
Resistance Source	region	association
PI71506	Rag1, Rag3	0.004, 0.0131
PI88508	Rag2	< 0.0001
PI437696	Rag1, Rag2	0.0045, <0.0001
PI507298	Rag2	< 0.0001
PI548237	Rag2	<0.0001
PI567391	Rag2	<0.0001
PI587656	Rag2	<0.0001
PI587663	Rag2	<0.0001
PI587775	Rag2	<0.0001
PI587870	Rag2	<0.0001
PI587899	Rag2	<0.0001
PI587972	Rag2	<0.0001
PI588000	Rag2	<0.0001
PI594573	Rag2	<0.0001
PI594707	Rag2	<0.0001
PI594822	Rag2	< 0.0001
PI594879	Rag2	<0.0001
PI599955	Rag2	<0.0001

## Identify Diversity in the Germplasm Collection Efficiently

- Can we predict which accessions have resistance at *Rag1* or *Rag2*?
- Germplasm collection
  being tested with 50,000
  SNP markers.



#### Haplotypes Across Rag2 Interval

Accession	Gene	SNPs	Haplotype
PI088508	Rag2	CCNGCAATAGG	1
PI243540	Rag2	TCCATGAGGGA	2
PI567391	Rag2	NNCATAGTAGN	3,4
PI587899	Rag2	NNCATNNTANN	3,4
PI587656	Rag2	TNCATAGTAGA	4
PI594822	Rag2	TNCATAGTAGG	3
PI507298	Rag2	TNCATGAGGGA	2
PI587775	Rag2	TNNATAGTAGG	3
PI587870	Rag2	TNNATAGTAGG	3
PI587972	Rag2	TNNATAGTAGG	3
PI588000	Rag2	TNNATAGTAGG	3
PI594707	Rag2	TNNATAGTAGG	3
PI594879	Rag2	TNNATAGTAGG	3



- None of the *Rag2* interval haplotypes are unique to only the aphid resistant genotypes.
- Suggests that greater marker density needed to predict the presence resistance at *Rag2*.

# Conclusions

- *Rag1* and *Rag2* mapped and fine mapped.
- Williams 82 sequence useful in fine mapping but did not lead to identifying gene candidates in the resistance source.
- Gene candidates for *Rag1* identified by cloning region from the resistance source.
- Aphid resistance gene in the *Rag2* interval found in 17 additional PIs.
- SNP haplotypes across the *Rag2* interval not predictive of aphid resistance in this region.

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PRODUCING RESULTS