

A photograph of a soybean field during harvest. The foreground and middle ground are filled with rows of mature, brown soybean plants. In the background, a combine harvester is visible, working in the field. The sky is overcast and grey.

Improving Soybean Using Exotic Germplasm

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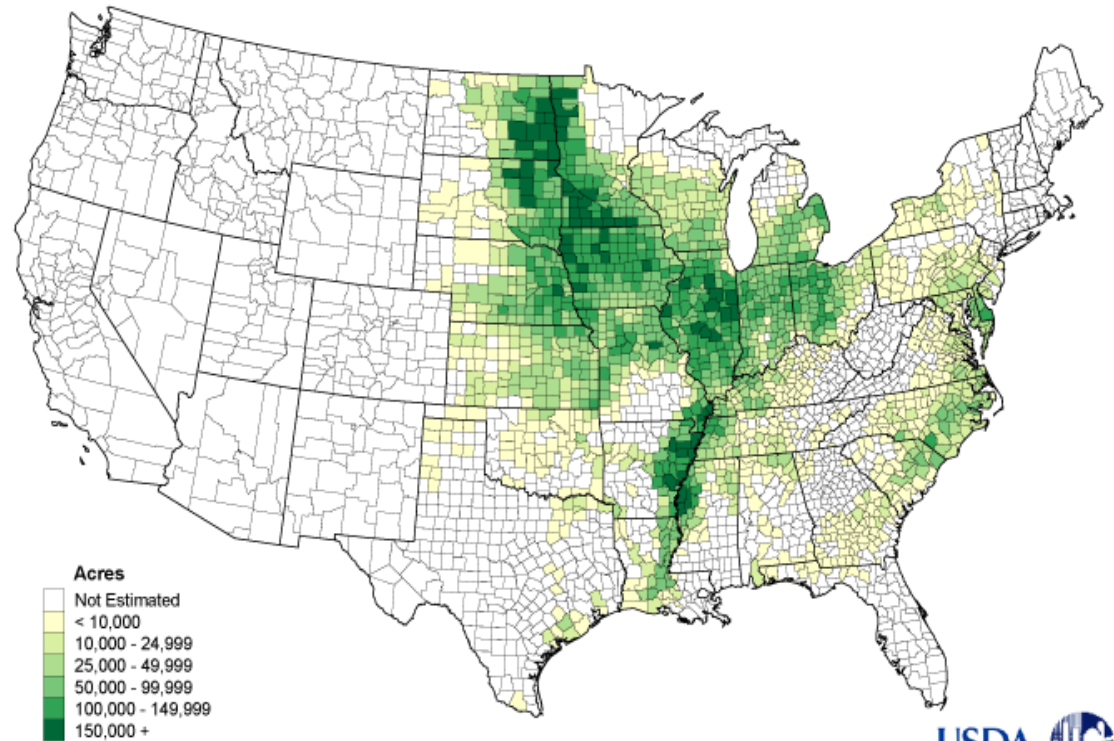
Outline

- Background on soybean.
 - US soybean production.
 - Soybean breeding programs.
 - Soybean genomic resources.
 - Soybean germplasm collections.
- Putting the germplasm and genomic resources together.
 - Soybean aphid resistance.
- New genetic technologies.

Background

Soybean 2004 Plant Acres

- Soybean grown on 29 million hectares in the US in 2008, primarily in the Midwestern US.
- Total US production in 2008 is projected to be 81 million metric tons.
- Farm value of 18 billion US dollars.



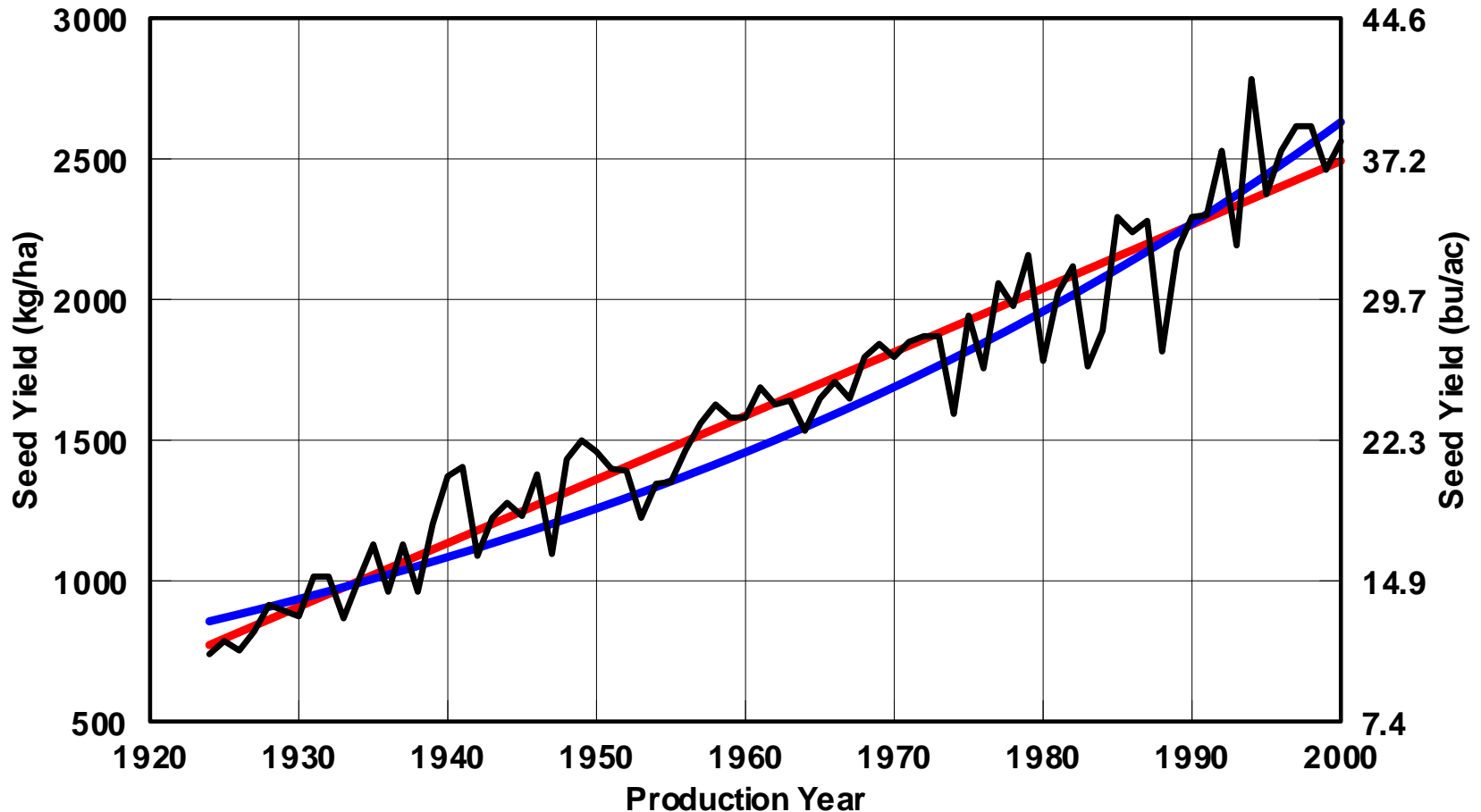
U.S. Department of Agriculture, National Agricultural Statistics Service



Background

- Soybean seed is multifunctional but is primarily a protein crop.
- Average 40% protein and 20% oil on a dry weight basis.
- Soybean seed is crushed for oil and the protein is used as a high protein feed.
 - Protein feed to poultry, swine, and cattle.

Soybean Yield Advancements from Genetic Gain and Improved Agronomics

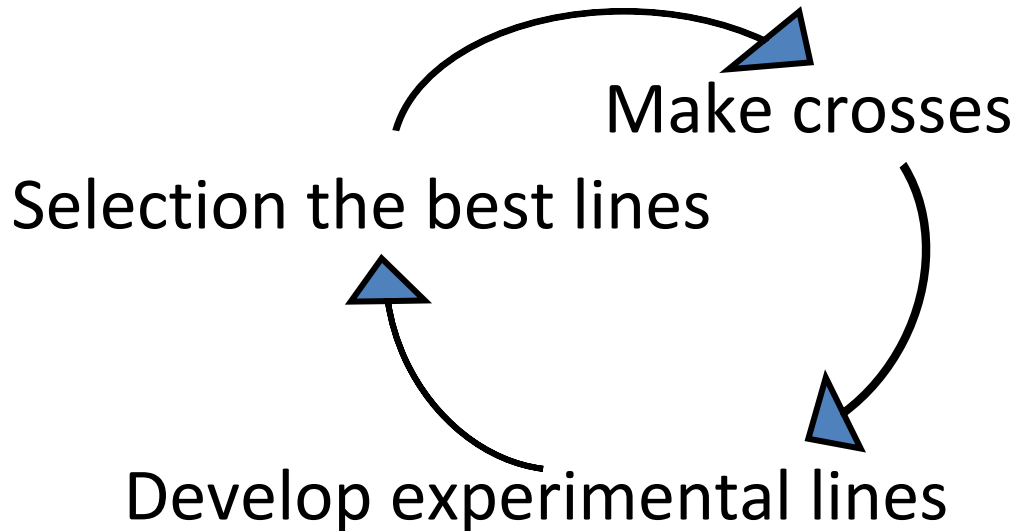


LIN: $b = 22.6\text{kg/ha}$ or 0.337bu/ac $R^2 = 0.934$ EXP: $b = 0.01464$ $R^2=0.927$

From Jim Specht et al., 1999 (Crop Sci. 39:1560-1570)

Soybean Breeding

- Most of the yield improvements of varieties are the result of phenotypic selection through traditional breeding.



Most Cultivars Grown are Developed by the Private Sector

- Private sector has over 90% of the soybean seed market.
- Private sector has an enormous enterprise in soybean breeding and biotechnology.
- Yield is the trait that receives the greatest focus.



Public Sector Research

- Public sectors is moving out of general use cultivar development.
- Focus of effort on:
 - Cultivars for specialty markets.
 - Germplasm development.
 - Identification of new genes from the germplasm collection.
 - Basic research.
 - Student educations.
- Research largely funded by farmer check off programs.



Impacts of Molecular Genetics on Soybean Breeding

- Large investment in molecular biology research has occurred.
 - Markers developed.
 - Marker assisted selection (MAS).
 - Transformation methods available.
 - Roundup Ready (herbicide tolerance).
 - Genome being sequenced.
 - Microarrays available for gene expression studies.

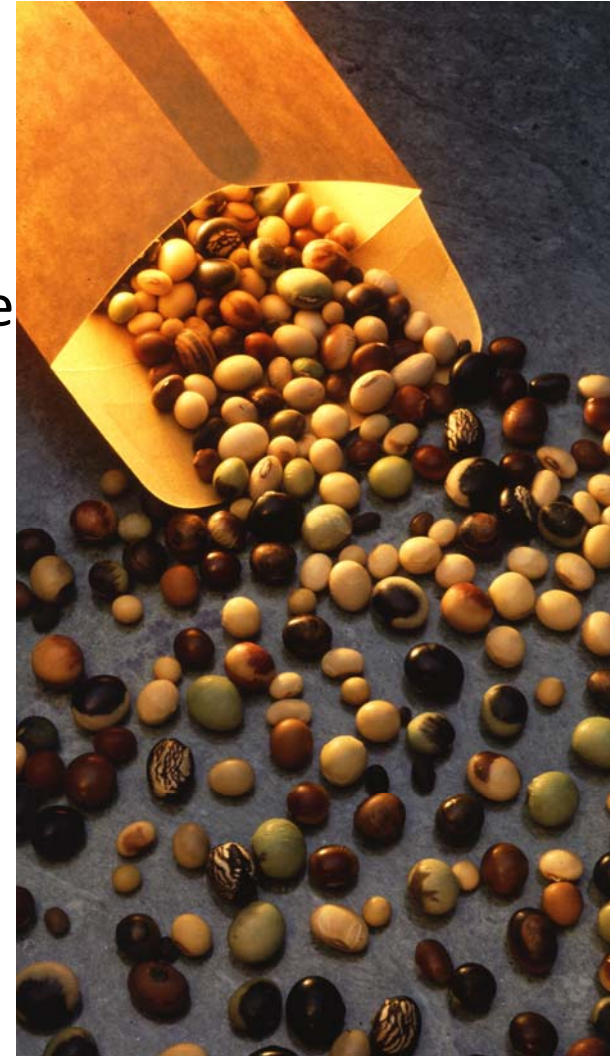
Soybean is Largely a GMO Crop

- GMO soybean have a Glyphosate tolerance (Roundup Ready - RR) gene developed by Monsanto.
 - The use RR soybean makes it the crop with the most genetically engineered acreage.
 - RR trait in 91% of soybean grown in the US (2007), 98+% of soybean in Argentina and a 53% in Brazil (2007-2008 growing season).
 - Farmers embraced this trait because it provided easy weed control.
- New RR genes will be released.



Soybean Genetic Diversity

- Elite North American Soybean germplasm has a narrow genetic base.
 - 80% can be traced to 13 ancestral lines.
 - 50% of the northern US germplasm can be traced to 3 ancestral lines.
- The USDA-ARS soybean germplasm collection includes:
 - 21,064 accessions.
 - 18,893 *Glycine max* (soybean).
 - 1,169 *Glycine soja* (wild annual soybean).
- Success in using germplasm for defensive traits.



Putting Germplasm and Genetic Resources Together - Soybean Aphid Resistance

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



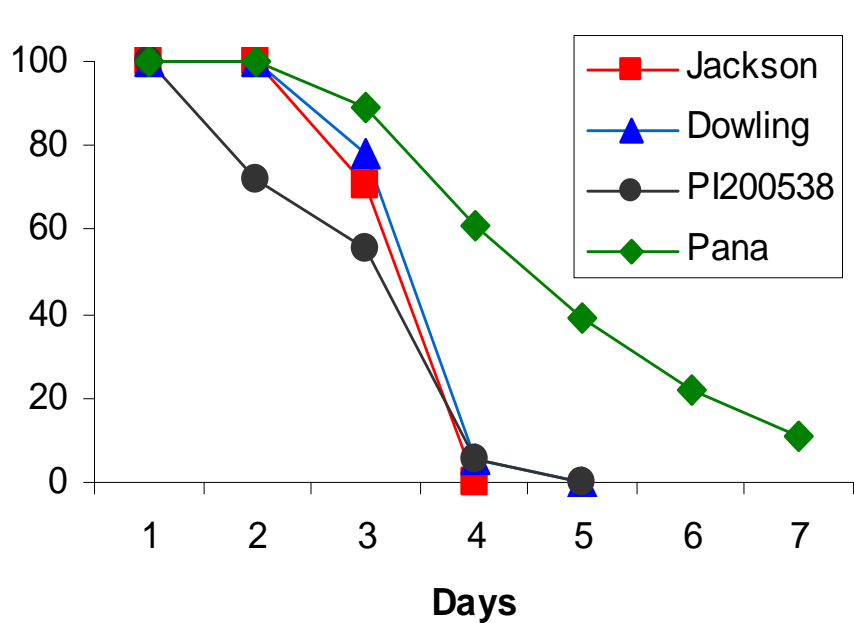
Screening for Soybean Aphid Resistance

Germplasm	Number tested	Number resistant
Commercial cultivars (MG II, II, IV)	818	0
Asian cultivars	106	0
Pubescent lines	11	0
Ancestral lines	101	9
Diverse collection of germplasm accessions	>3000	85

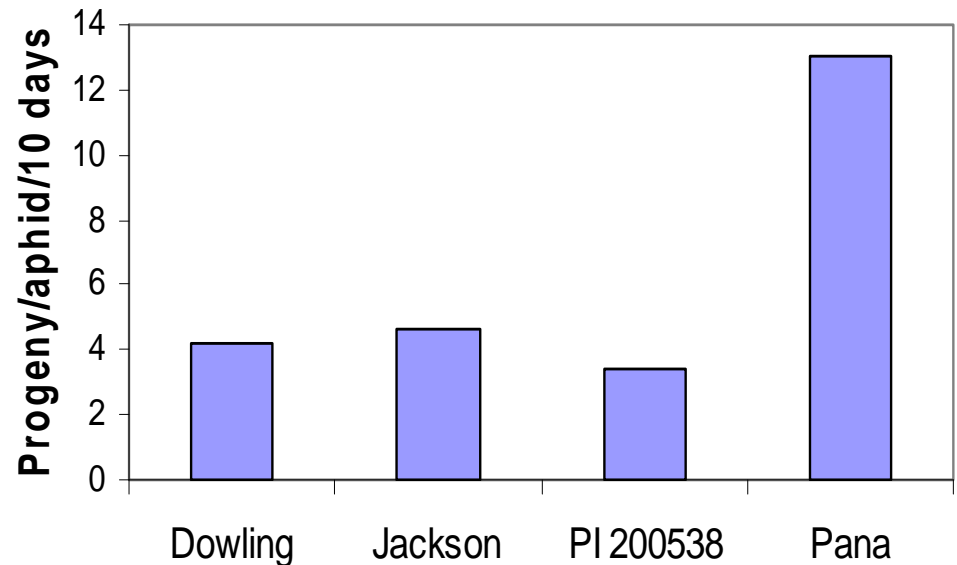
Hill, C.B., Y. Li, and G. L. Hartman. 2004. Resistance to the soybean aphid in soybean germplasm. *Crop Science* 44: 98-106.

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.

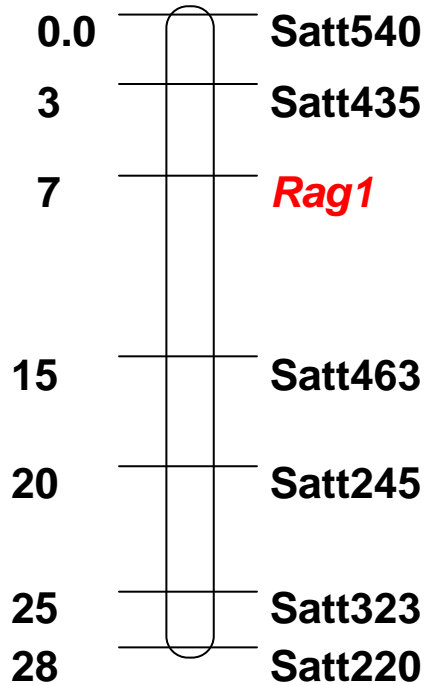
Mapping Aphid Resistance

- The maturities of Dowling (MG VIII), Jackson (MG VII) and PI 200538 (MG VIII) make them incompatible for production in the Midwest.
- The resistance gene(s) need to be bred into northern soybean backgrounds.
- These genes were mapped quickly with SSR markers using bulked segregant analysis.

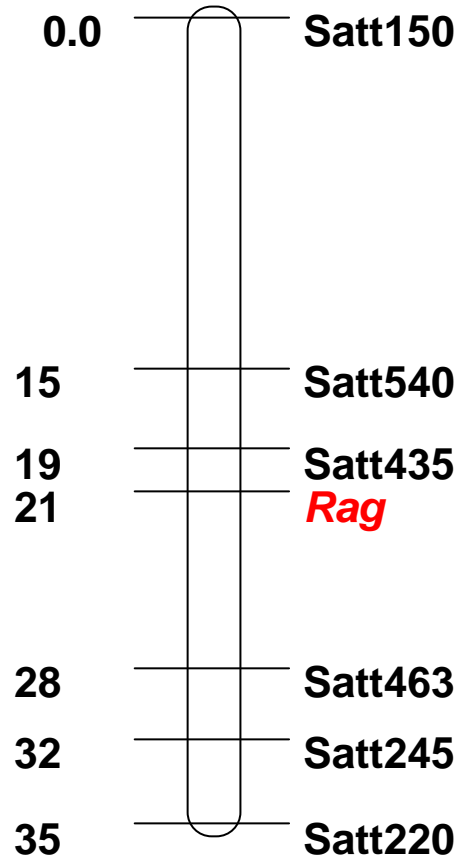


Map Location of the Aphid Resistance Genes *Rag1* and *Rag2*

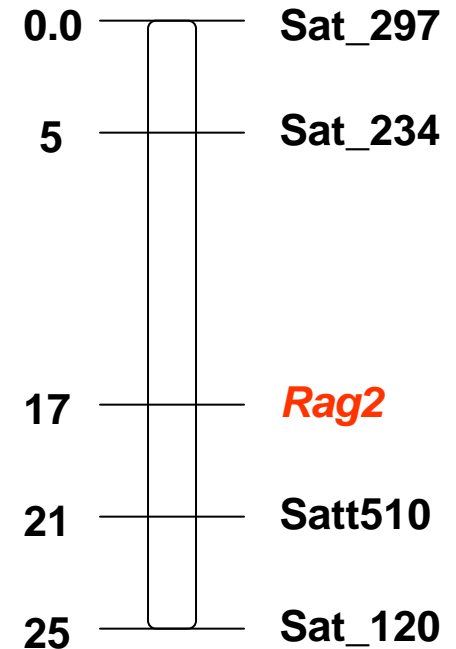
Dowling *Rag1*
LG M



Jackson *Rag*
LG M

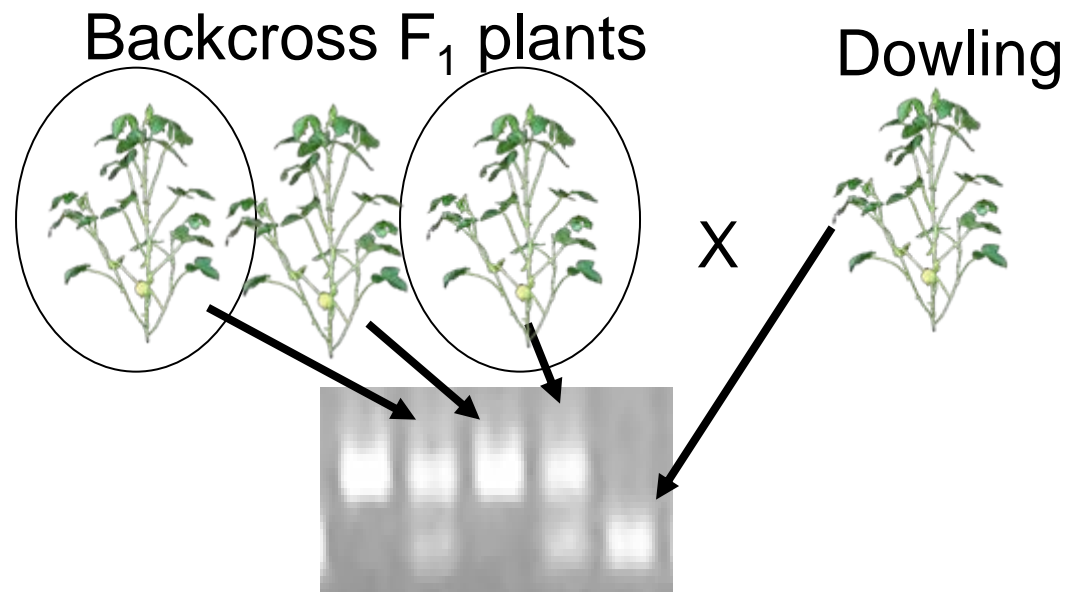


PI 200538
Rag2 LG M



Backcrossing Aphid Resistance

- In 16 months four backcrosses were made to move the *Rag1* gene from Dowling into the background of the MG II variety Loda.
 - Developed BC4F₁ plants in 3 other backgrounds.





Aphid Resistant Line

Aphid Susceptible Line

Aphid Infestations

Resistant Line



Susceptible Line



Aphid Resistance

Matt O'Neal, Iowa State University

Soybean aphid resistance tested on naturally occurring populations.

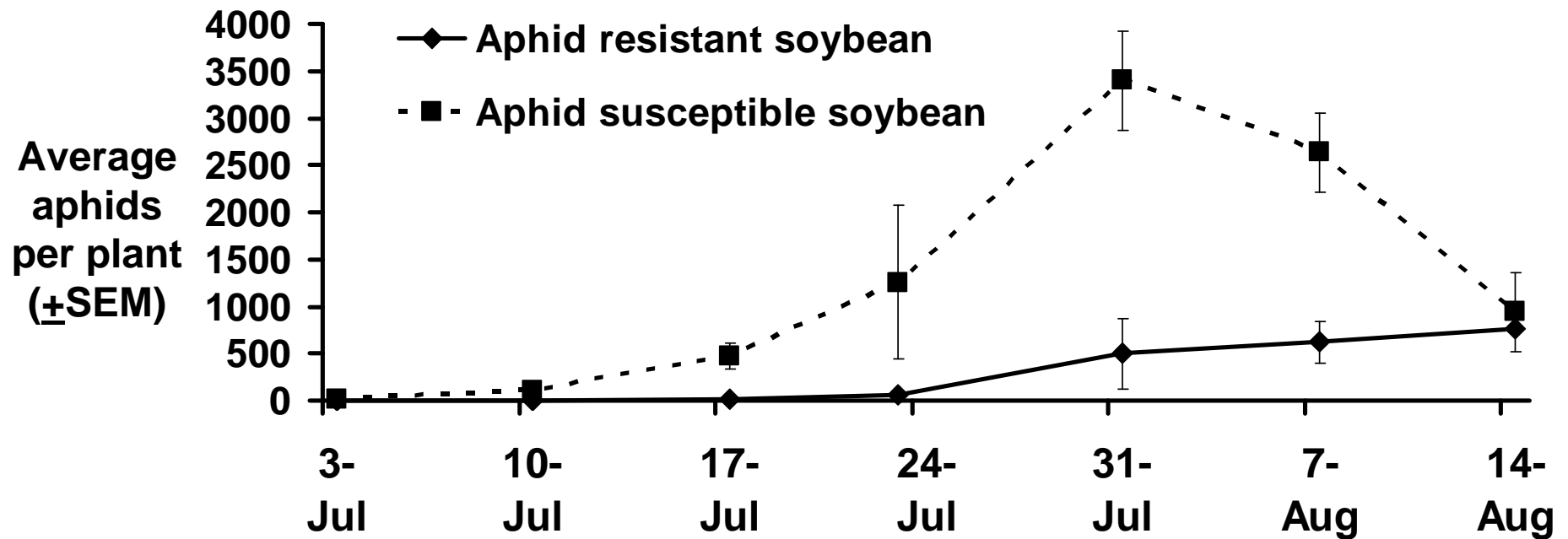


Resistant soybeans were not aphid free, reaching threshold by 31 July 2007.

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Aphid Biotypes

	Aphid Isolate	
	Illinois	Ohio
	---No. of Aphids Plant ⁻¹ ---	
Dwight (Susceptible)	468	398
Williams 82 (Susceptible)	726	574
Dowling (<i>Rag1</i>)	15	639
LD05-16611(<i>Rag1</i>)	29	627
PI 200538 (<i>Rag2</i>)	10	7
PI 567597C (MSU)	21	7

What Next for Aphid Resistance?

- Continuing with the release of cultivars with *Rag1*.
 - Gene has not broken down in field tests.
- Fine mapping *Rag1* with a goal of cloning it.
- Developing cultivars with *Rag2* from PI 200538.
- Studying the genetic basis of resistance in more PIs.



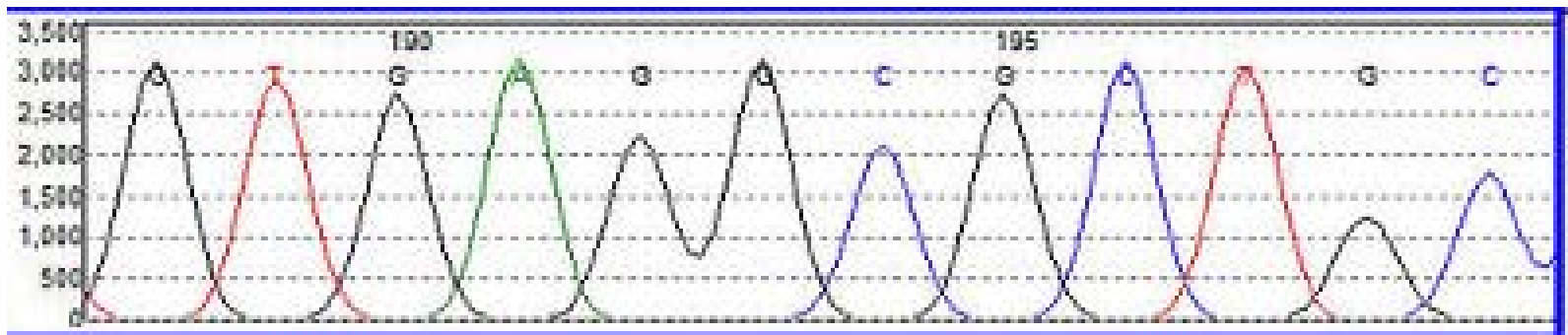
New Technology in Soybean Improvement

- Genomic research is impacting soybean improvement and these impacts will accelerate in the future.
 - High throughput sequencing.
 - Cheaper and better markers.



Sequencing of the Soybean Genome

- Soybean variety Williams 82 is being sequenced.
 - US Department of Energy-Joint Genome Institute has completed a 7X shotgun sequence of the soybean genome using Sanger sequencing methods.
- Soybean genome is approximately 950 Mb.
- Sequence arranged in supercontigs averaging 6.5 Mb.



High Throughput Resequencing

- Having the reference Williams 82 sequence opens doors to resequencing using high throughput methods.
- Resequencing is used to identify single nucleotide polymorphism (SNP) markers and allelic diversity.



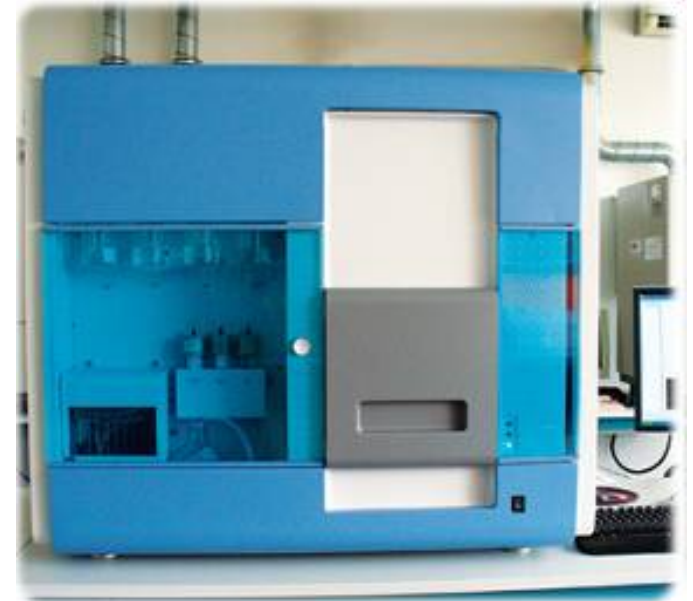
"The scaffold has been handed down to us from our ancestors, and through it we are connected to all other life on earth."

- Svante Pääbo

Revolution Through Competition.

Illumina (Solexa) Sequencer

- Solexa sequencer can sequence 1.5-2 billion bp of DNA for \$7,000 US in 3 days (plus cost of library).
- Reads are 36 bp either singly or in pairs.
- Promise 50 bp reads by end of the year and 100 bp reads eventually.



Fine Mapping *Rag1*

- Genome sequence being used to fine map *Rag1*.
 - Identify markers closely linked to the gene and eventually clone it.
- *Rag1* interval placed on a 1.7 million bp contig.
- Tested 2,000 backcross F₂ plants with segregating markers to identify recombinants near the gene.
- Developed new markers near *Rag1* using genome sequence.

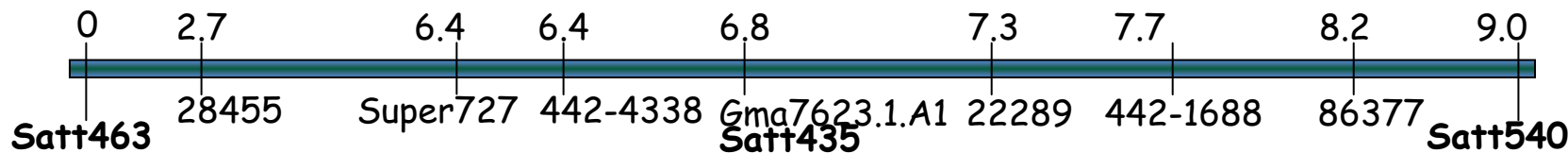
Primer tiling

64 Total Primer Pairs

- 34 Pairs Worked for PCR
- 23 Used for Sequencing
- 10/23 Used for Successful Sequencing
- Found 8 SNPs

Fine Mapping *Rag1*

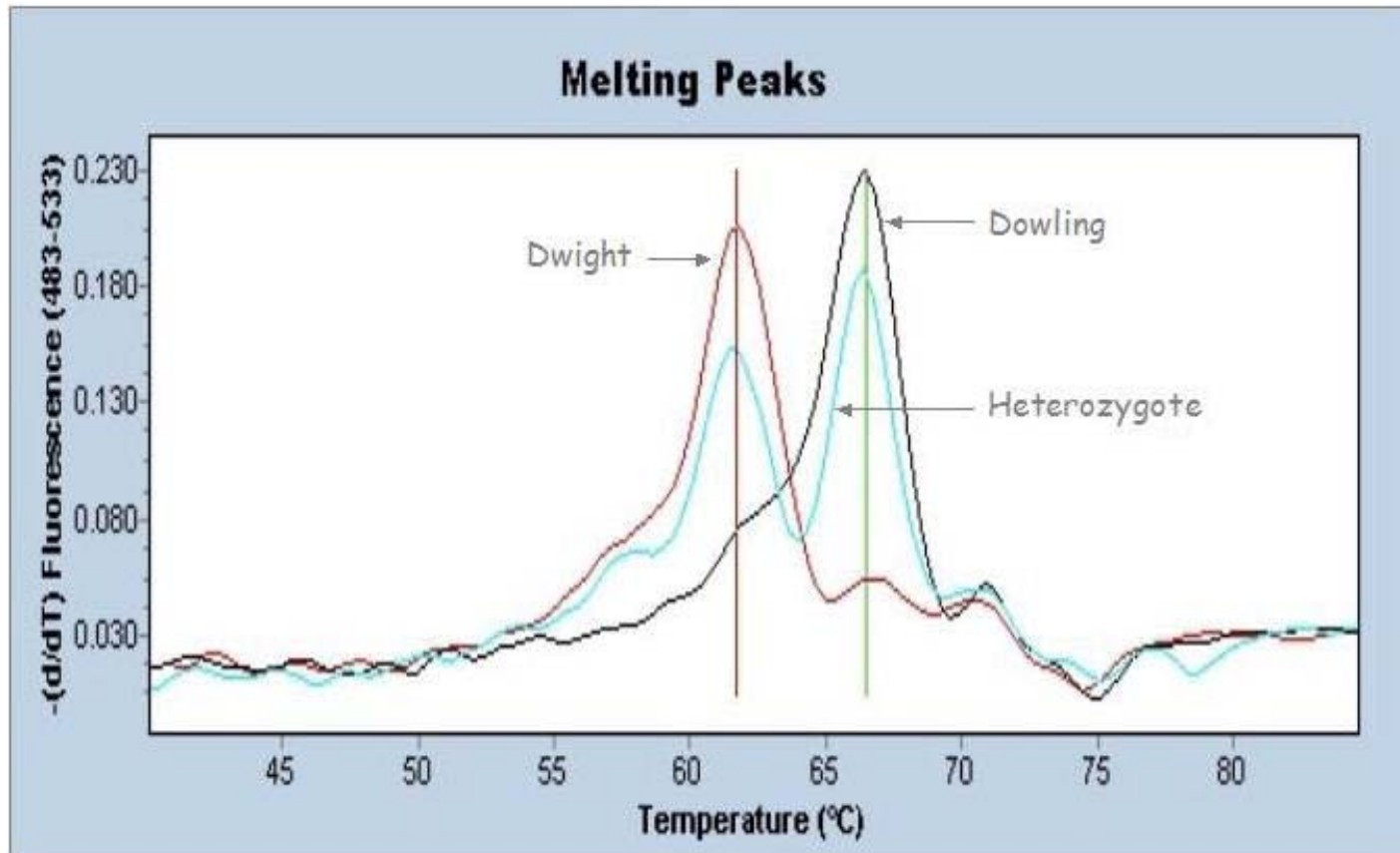
- Testing the resistance of lines derived from recombinant plants.
- Mapped the gene to a 150,000 bp interval.



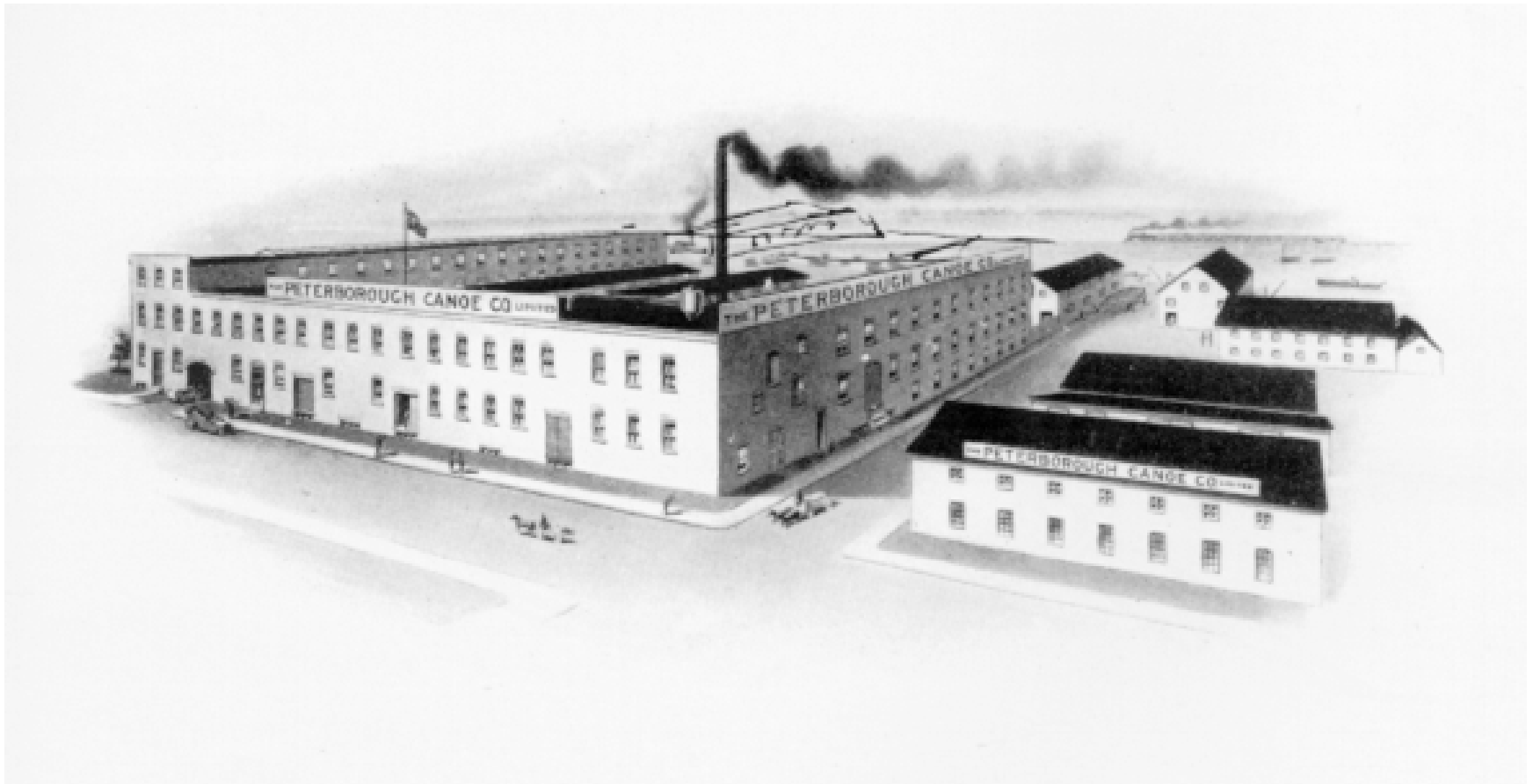
Genetic distance (cM)	0	2.785	-	6.384	6.388	-	-	-	-	6.842	6.842	7.285	
Physical distance (bp)	-	198,879	406,545	442,852	472,667	505,610	558,337	571,766	583,892	586,788	629,590	822,688	
DNA #	Phenotype	Satt463	28455	27A	Super727	442-4338	56B	21A	25A	83A	Satt435	7623.1.A1	22289
12	Segregation	B	B	H	H	H					H	H	H
72	Segregation	B	B	H	H	H					H	H	H
4	Resistance	H	H	H	A	A					A	A	A
82	Resistance	A	A		A	A	A	H	H	H	H	H	H
100	Segregation	H	H		H	H	H	A	A	A	A	A	A
6	Segregation	H	H		H	H	H	H	H	H	A	A	A
48	Susceptible	B	B		B	B					B	H	H
7	Resistance	A	A		A	A					A	A	H
62	Resistance	A	A		A	A					A	A	H
73	Resistance	A	A		A	A					A	A	A
92	Resistance	A	A		A	A					A	A	A

SNP Markers for MAS

- TaqMan or melting curve assays being done to select *Rag1*.
- Testing plants on the order of 10,000 genotypes annually.



MAS in soybean is being done on an industrial scale in private industry



Impacts of Molecular Genetics on Soybean Breeding

- Marker-assisted selection (MAS).
 - Pioneer Hi-Bred will test close to 2 million samples with markers in 2006, most on soybean.
 - Monsanto also has a large effort.
 - Most MAS in soybean is being done for defensive traits, such as disease resistance.
 - Pioneer is now using MAS for yield.

High Throughput Marker Analysis

- Illumina Golden Gate assays have been designed for soybean.
 - Can test 1,536 SNPs on 96 genotypes in 2 days.
 - High throughput methods that is useful for the discovery of gene locations in mapping populations.



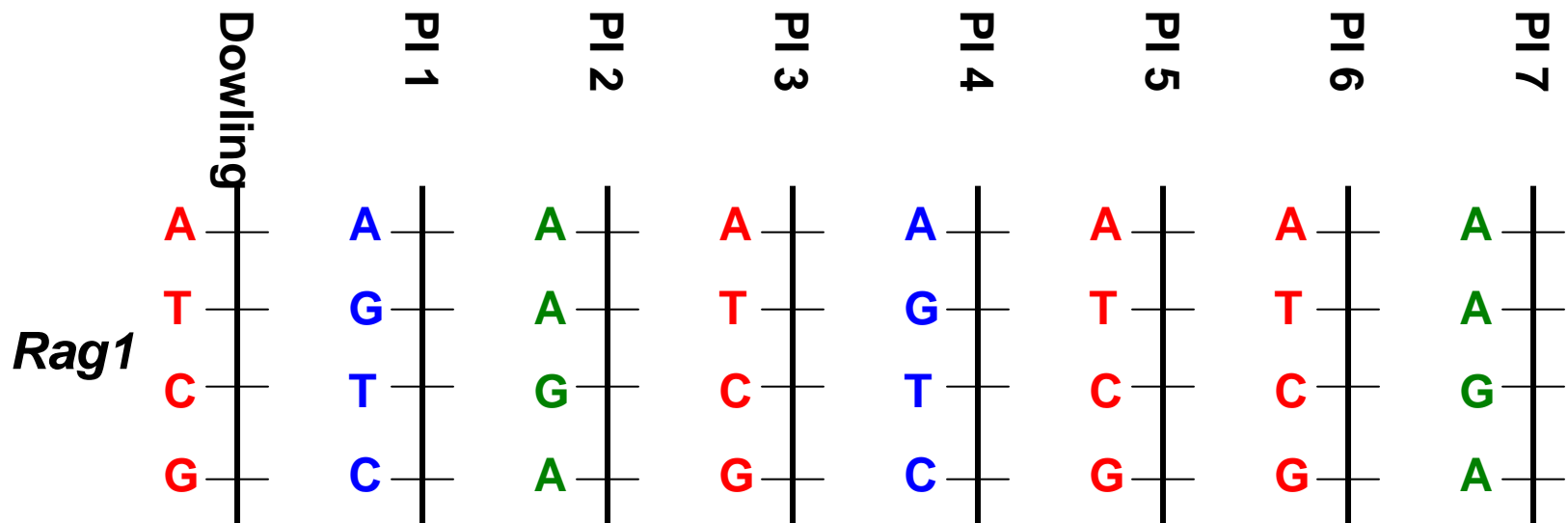
Bead Station 500

High Throughput Marker Analysis

- Illumina Infinium assays being developed for soybean.
 - Test over 40,000 SNPs / genotype.
 - Funding to test all accessions in the USDA germplasm collection has been secured.
 - Use markers in identify unique germplasm, conduct association analysis, compare regions where important genes are located.

High Throughput Marker Analysis

- There are 80 PIs with aphid resistance.
- Which most likely have new resistance genes?
- Compare SNPs across gene containing region.
 - Resistant PIs with unique genotypes likely to have different resistance genes.



Conclusions

- Soybean improvement is continuing.
- Most yield improvements to date are the result of traditional breeding.
- Most cultivars are developed by the private sector.
 - These are glyphosate tolerant GMOs.
- Public sector is focused on specialty varieties and research.
- North American soybean germplasm has a narrow genetic base.

Conclusions

- Sources of soybean aphid resistance identified.
 - Resistance is antibiosis.
- Mapped resistance genes from 3 aphid resistance sources.
- Aphid resistant varieties develop through marker assisted selection.
- Aphid biotype diversity found.

Conclusions

- New genetic technology will speed soybean improvement.
- Soybean genome sequenced.
- New technology will allow rapid resequencing and marker development.
- Fine mapping *Rag1* aphid resistance gene.
- Marker technology developed for MAS.
- Marker technology developed for genome saturation.

Acknowledgments

- Research was supported by:
 - United Soybean Board.
 - Illinois Soybean Checkoff Board.
 - USDA-NRI competitive grant program.

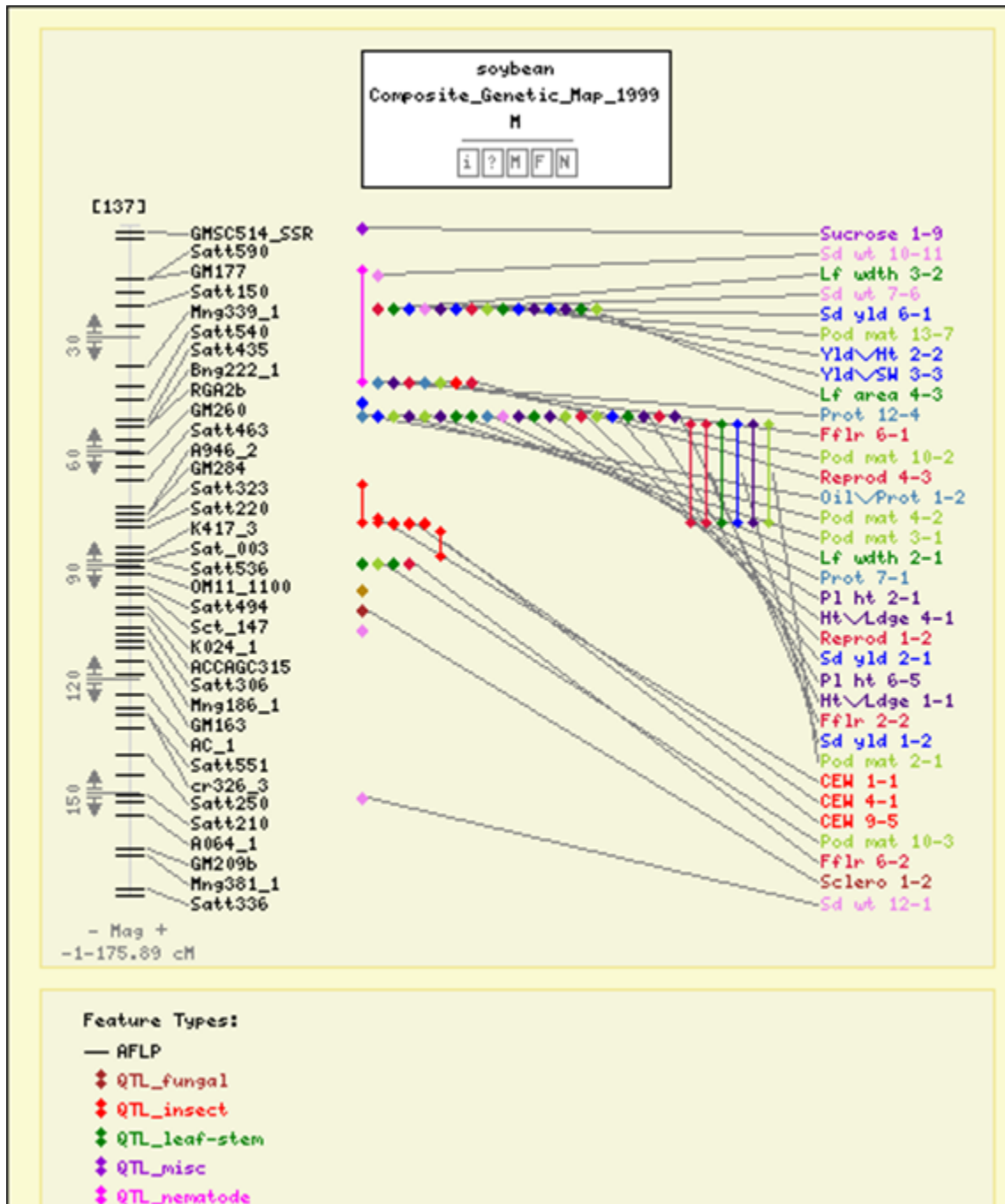


PRODUCING RESULTS

Impacts of Molecular Genetics on Soybean Breeding

- Marker-assisted selection (MAS) is widely used in private and public soybean breeding.
- Soybean has good genetic marker resources.
 - 1,000 microsatellite markers mapped.
 - 10,000 + SNP markers mapped.
- Many genes and QTL have been mapped.
 - Genes and QTL controlling resistance to important diseases.
 - QTL controlling yield and agronomic traits.

Mapped Soybean Genes and QTL



Aphid Resistance: The *Bad News*

- Aphid biotypes exist that can overcome *Rag1*.
 - Biotype = an insect population that is resistant to the defenses of the host plant.
 - *Rag1* defeating biotype found in Ohio.
 - Unofficial reports of these isolates in other locations.
- Biotype diversity expected eventually as observed for other aphid pests.
 - Multiple biotype found for Russian wheat aphid and greenbug.

The \$10 million X PRIZE for Genomics prize purse will be awarded to the first Team that can build a device and use it to sequence 100 human genomes within 10 days or less, with an accuracy of no more than one error in every 100,000 bases sequenced, with sequences accurately covering at least 98% of the genome, and at a recurring cost of no more than \$10,000 per genome.

Aphid Infested Field Cage Test

- Tested the equivalent of BC1F₂-derived resistant and susceptible lines in field cages.

Trait	Res.	Susc.
Aphid rating (6-28)	0.8	2.3
Aphid rating (7-22)	1.6	3.3
Plant height (inches)	35.8	30.0
Seed yield (g)	79.9	19.2

Aphid rating: 0 = no aphids, 4 = severe plant damage.
Difference between resistant and susceptible genotypes significant for all traits ($P < 0.05$).