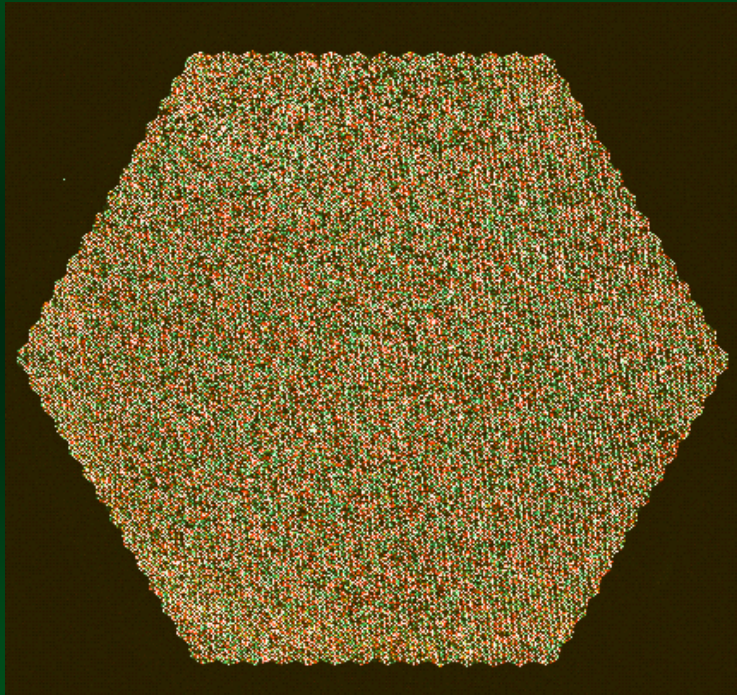


Mapping Soybean Rust Single Gene Resistance

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Agriculture
Research, Education &
Economics
Agricultural Research
Service

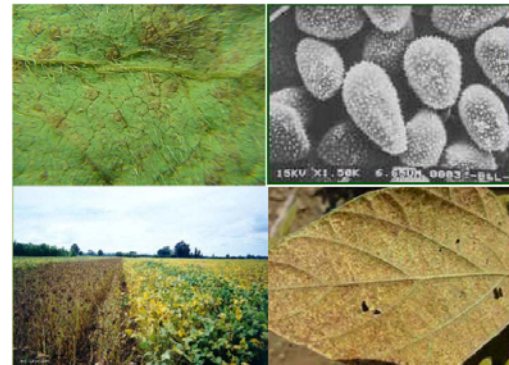
National Program Staff

April, 2005

Version 1.3

National Strategic Plan for the Coordination and Integration of Soybean Rust Research

Asian Soybean Rust
Phakopsora pachyrhizi



United States Department of Agriculture
Agricultural Research Service



Beltsville Area / Soybean Genomics and Improvement Laboratory

Single Gene Resistance

- Four resistant genes to the fungus *Phakopsora pachyrhizi* identified between 1980 and 1986
 - Identified with foreign isolates from India, Australia, Philippines, and Taiwan
 - Single dominant genes
 - *Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4*
 - Requires Biosafety Level 3 containment
 - USDA-ARS Foreign Disease-Weed Science Research Unit at Ft. Detrick, Frederick, MD



Mapping *Rpp1*

- Williams 82 isoline of *Rpp1* available
 - 98.4% identical to Williams 82
- Found candidate regions with molecular markers
- Phenotyped and genotyped a segregating population
 - *Rpp1* mapped to linkage group G near Sct_187

Hyten et al. 2006, Crop Sci. 47:837-840



Williams 82
isoline
Resistant



Williams 82
Susceptible



Additional Mapped Rust Single Gene Resistance

- ***Rpp?*(*Hyuuga*)**
 - Linkage group (LG) C2
 - Satt460 – Sat_263. (Monteros et al. 2006, Crop Sci. 829-836)
- ***Rpp2***
 - LG J
 - Sat_255 – Satt620 (Abdelnoor et al. 2007, Plant and Animal Genomes XV Conf, poster 413)
- ***Rpp4***
 - LG G
 - Satt288 – Satt191, 30cM from *Rpp1* (Abdelnoor et al. 2007, Plant and Animal Genomes XV Conf, poster 413)



Outline

- **Bulk Segregate Analysis using GoldenGate assay**
 - Confirmation of *Rpp4*
 - *Rpp3*
 - Genotype population with SSR markers in candidate regions
- **Fine map using soybean genome sequence**
 - *Rpp1* and *Rpp3*



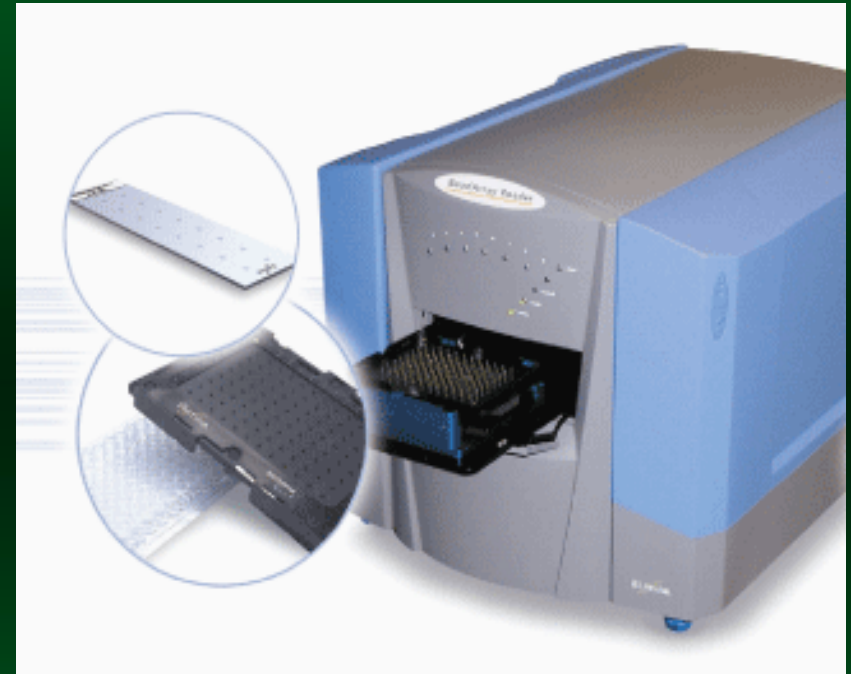
Bulked Segregate Analysis

- *Rpp4* isoline L87-0482 was segregating for resistance
- Planted 50 individual plants from isoline
- Isolated DNA then tested plants for rust resistance
 - 20 red brown (resistant)
 - 19 tan (susceptible)
- Created two resistance and susceptible bulks for isoline
- Tested bulks using GoldenGate assay on SoyOPA-2

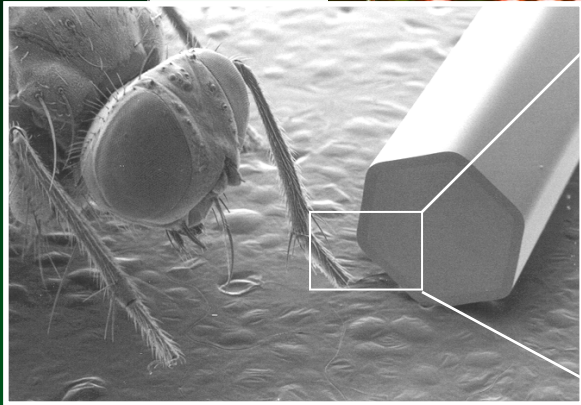
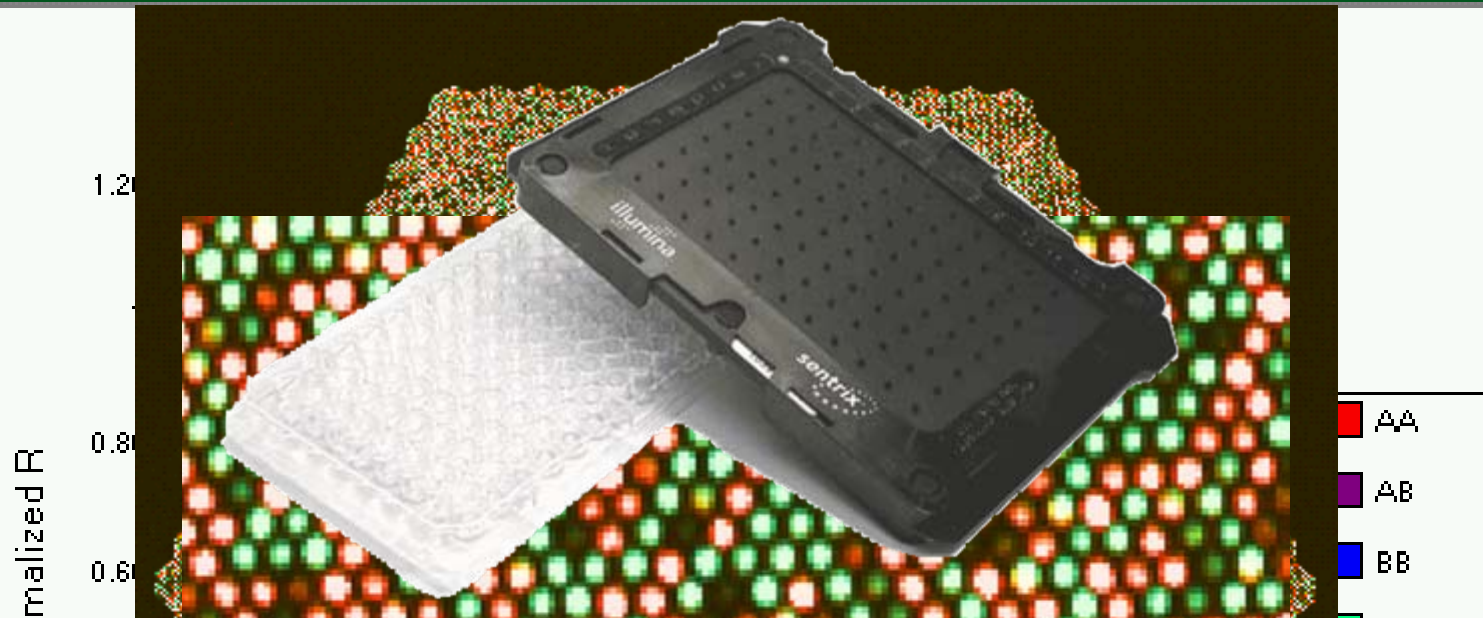


Illumina Beadstation 500

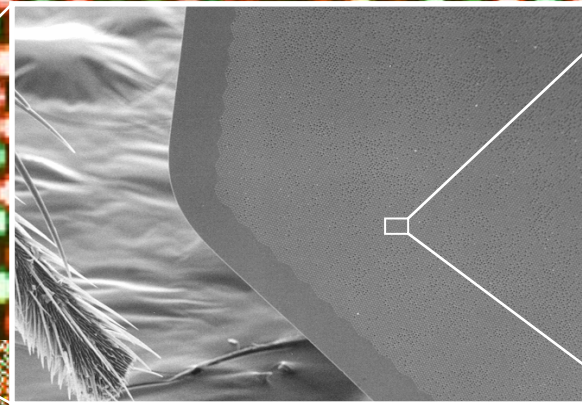
- Custom genotypes:
96-1,536 SNPs
- Total # DNA samples:
96-192 after 3 days
- Currently have tested
a 384 SNP array
(SoyOPA-1), and two
1536 SNP array
(**SoyOPA-2** and 3)
- Designed a universal 1,536 linkage panel by
selecting the best SNPs from previous arrays



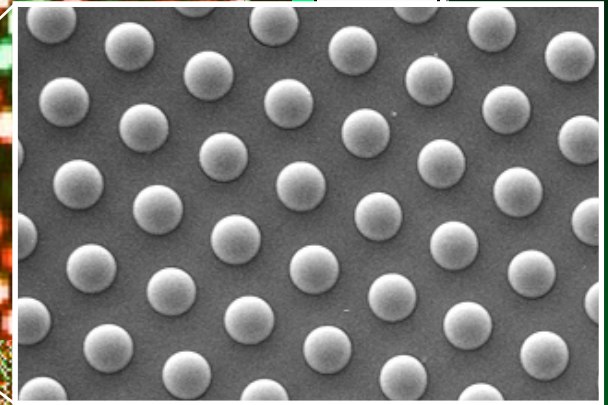
Sentrix[®] Array Matrix



1.5 mm



400 μ m

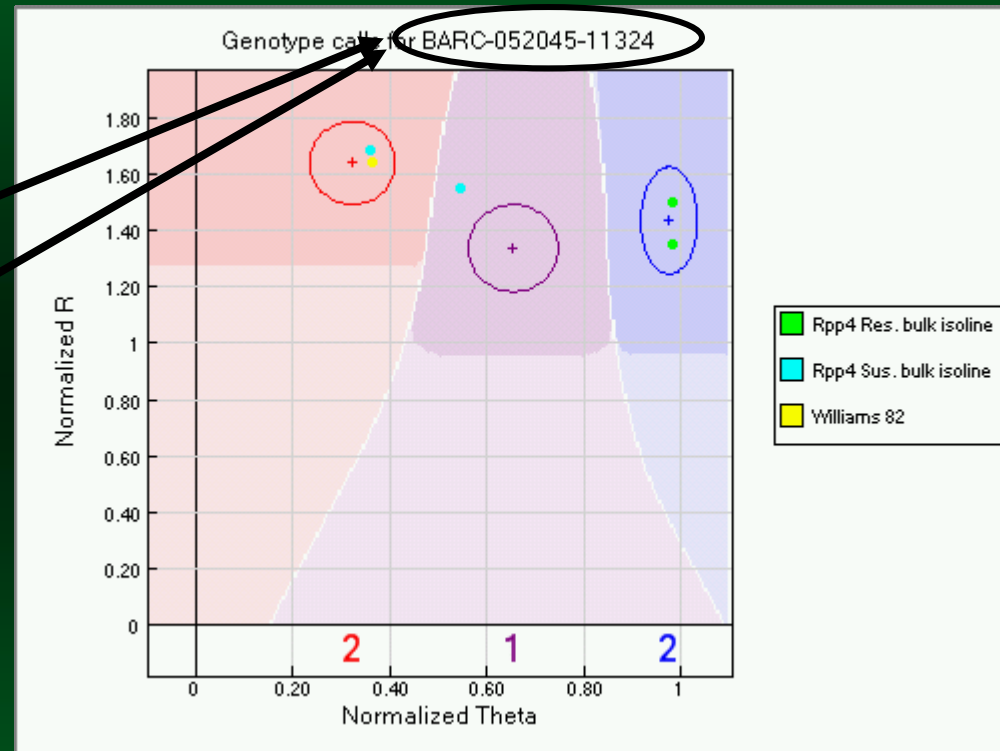
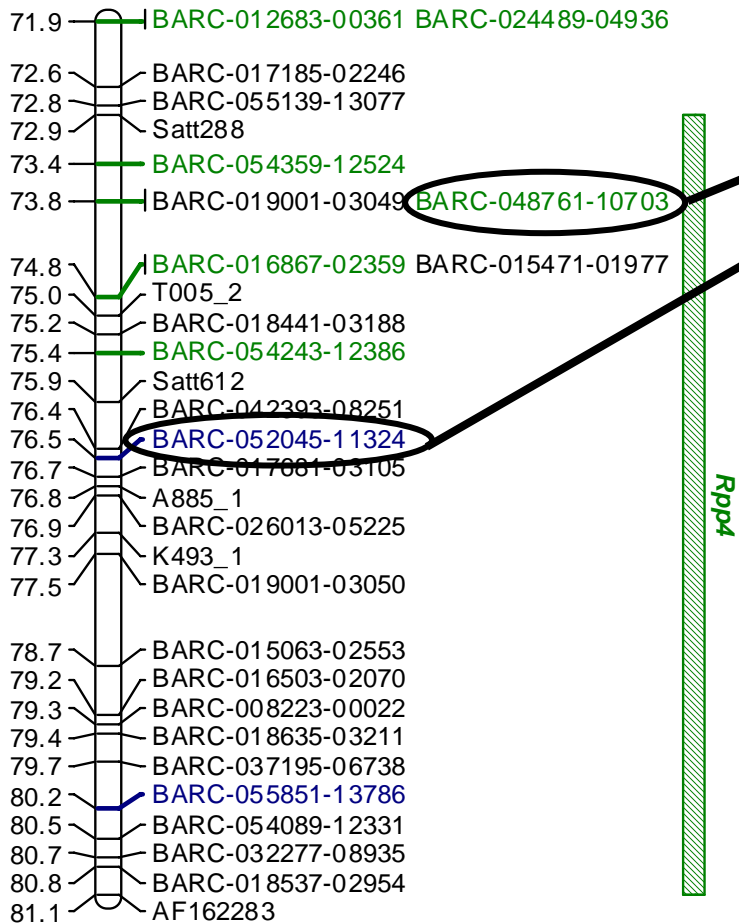


10 μ m



Confirmation of *Rpp4*

Linkage Group G



Abdelnoor et al. 2007

Mapping of *Rpp3*

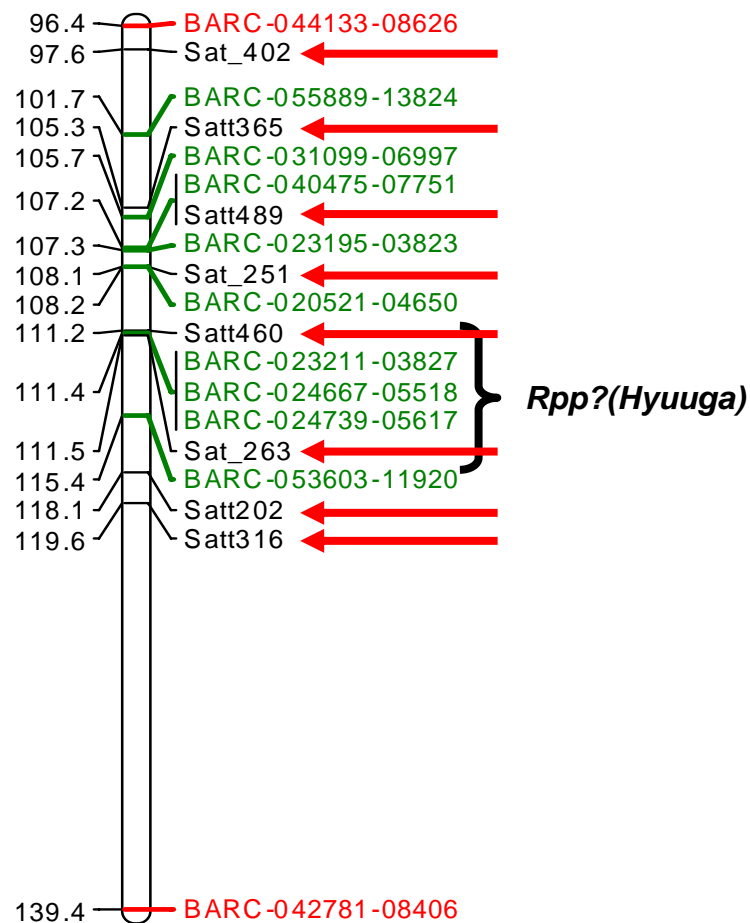
- 110 F_{2:3} lines from Williams 82 x PI 462312
- Screened for resistance with at Ft. Detrick, MD with India 73-1 isolate.
- Created one resistance and susceptible bulk
- Tested bulks using GoldenGate assay on SoyOPA-2



BSA position of *Rpp3*

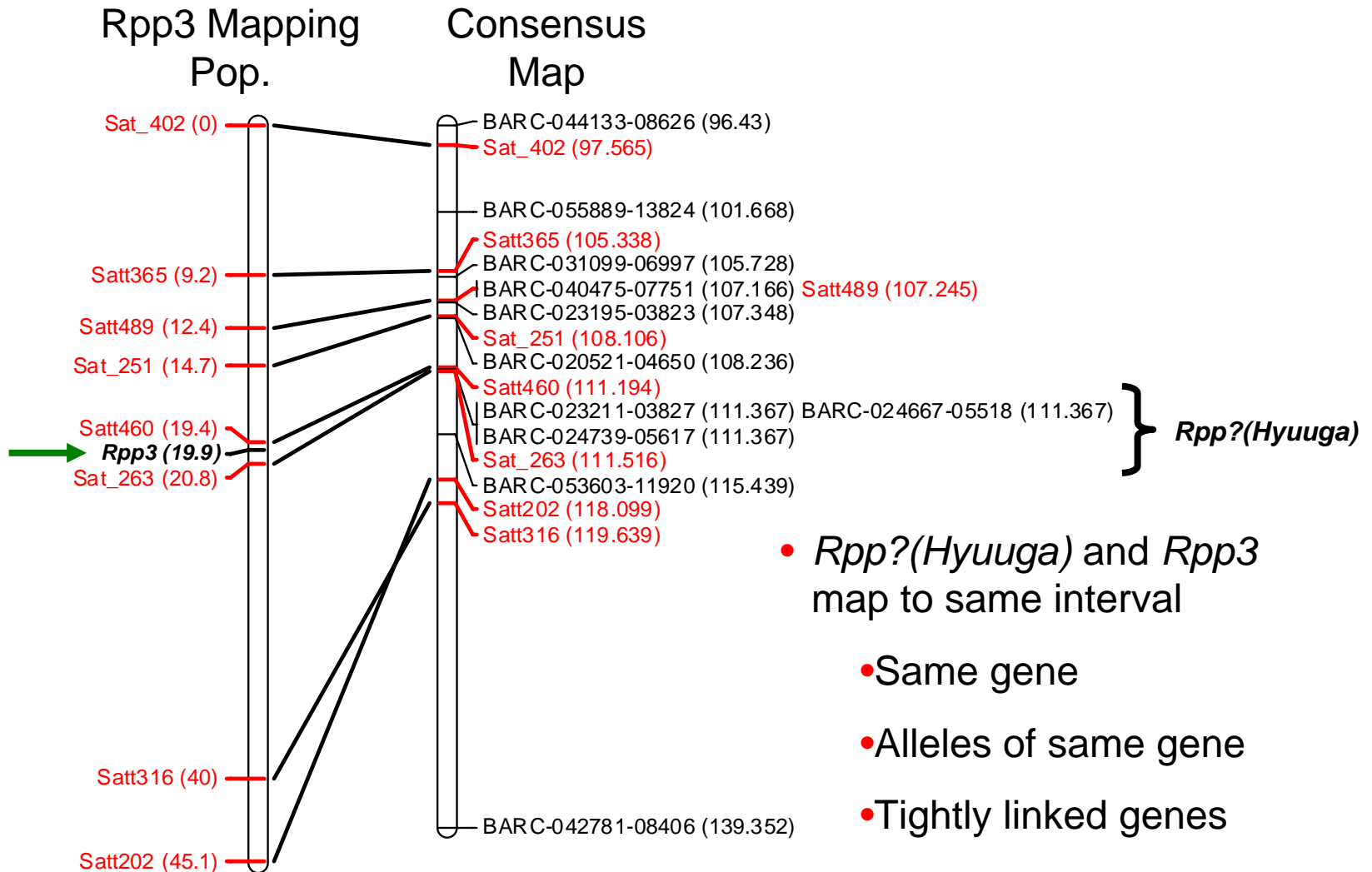
- Total of 27 SNPs positive for *Rpp3* from BSA
- All mapped to LG C2 between Sat_402-Satt202
- Genotyped 10 plants from each of the 110 $F_{2:3}$ families with SSR markers

Linkage Group C2



Map Position of *Rpp3*

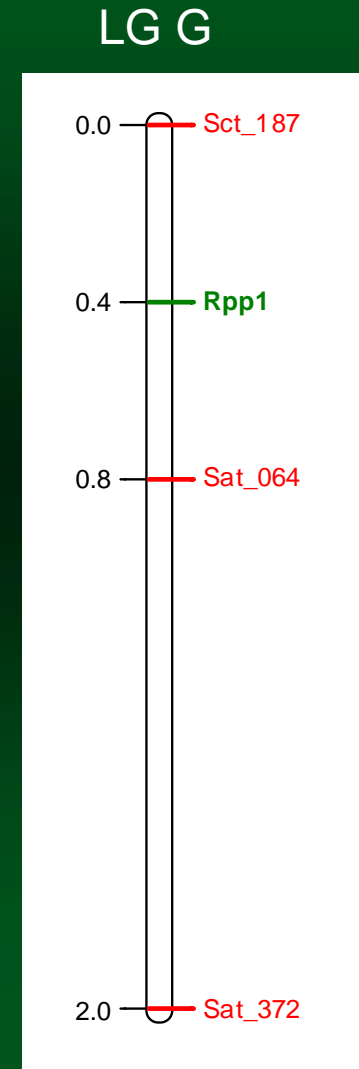
Linkage Group C2



- *Rpp?(Hyuuga)* and *Rpp3* map to same interval
 - Same gene
 - Alleles of same gene
 - Tightly linked genes

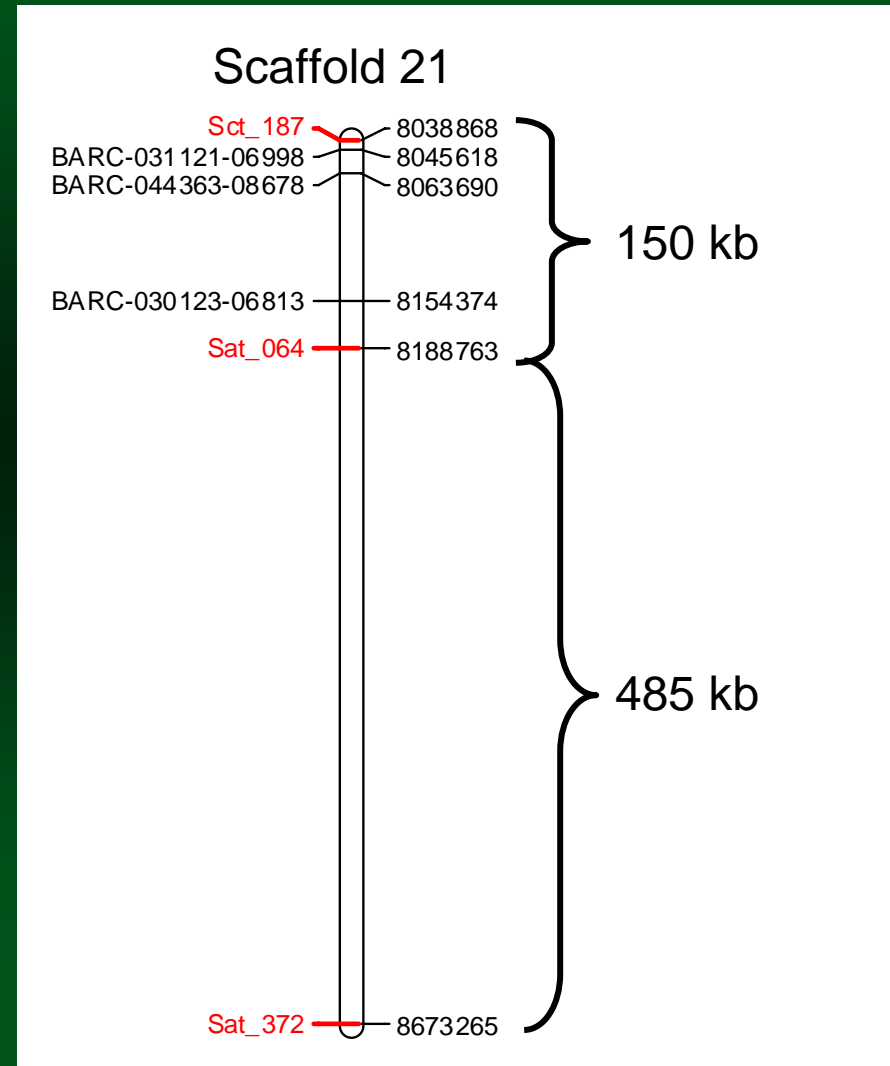
Fine Mapping of *Rpp1*

- Screened 1,091 F₄ plants for recombinants between Sct_187 and Sat_372
- 76 identified and screened for rust resistance at Ft. Detrick



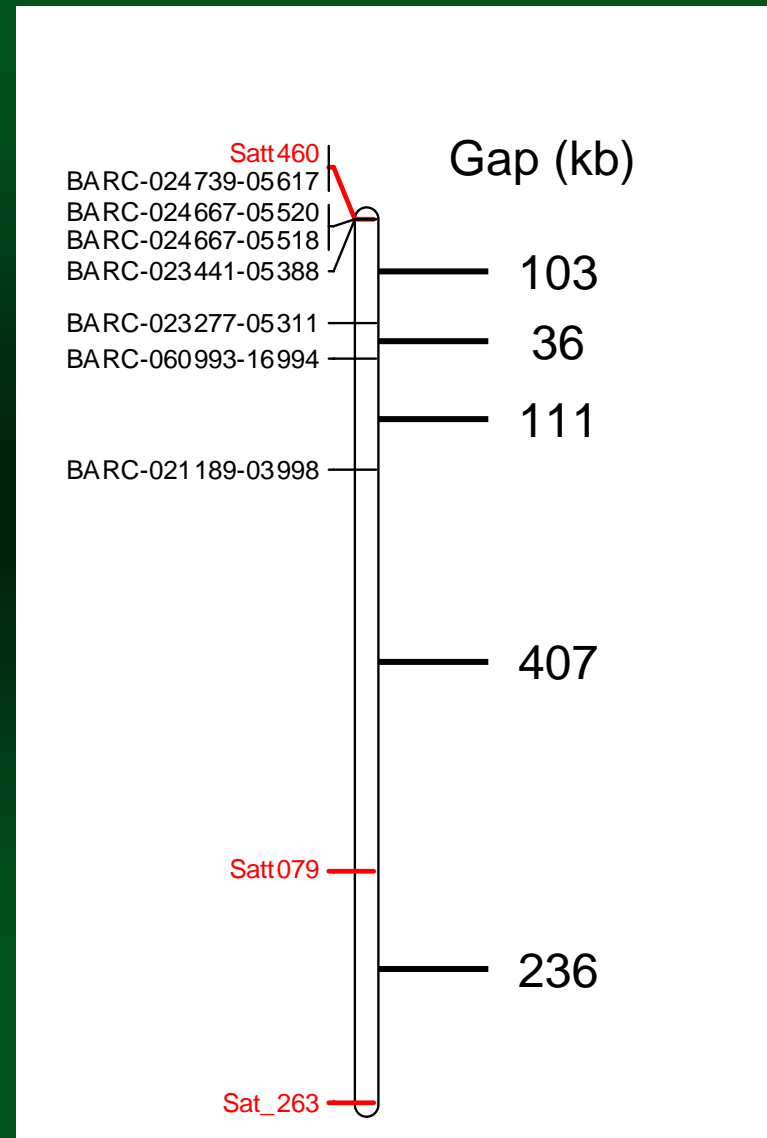
DOE-JGI Shotgun Sequence of Williams 82

- Scaffold 21 of the JGI sequence contains *Rpp1* mapped to a 150 kb region
- 3 SNPs located in *Rpp1* region
 - 91kb gap remains
- Designed 48 primer pairs distributed in region for screening 76 recombinants
- 16 recombinants between Sct_187 and *Rpp1* phenotype



Rpp3 Genomic Location

- Located on JGI scaffold 60
- Gene positioned in 897 kb gap
- Identified 7 recombinant plants between Satt460 and Sat_263
- Identify which gap *Rpp3* is located
 - Design additional primers
 - Screen for additional recombinants



Known Resistance Genes

Source	Gene	Linkage Group	Location	Comment
PI 084674	QTL	A1	Sat_137	Slow rusting
PI 462312	<i>Rpp3</i>	C2	Satt460	Mapped to 900 kb
Hyuuga	<i>Rpp?(Hyuuga)</i>	C2	Satt460	Same position as <i>Rpp3</i>
PI 578457 a	Single Gene	C2	Satt460	Same position as <i>Rpp3</i>
PI 459025 b	<i>Rpp4</i>	G	Satt288	30cM from <i>Rpp1</i>
PI 200492	<i>Rpp1</i>	G	Sct_187	Mapped to 150 kb
PI 594538 a	Single Gene	G	Sct_187	Res. to different strains than <i>Rpp1</i>
PI 084674	QTL	H	Sat_218	Slow Rusting
PI 230970	<i>Rpp2</i>	J	Not confirmed	
PI 084674	QTL	K	Satt628	Slow Rusting

Future Directions

- **Continue to fine map *Rpp1* and *Rpp3* to determine gene responsible for resistance**
 - May lead to solutions for more durable resistance
- **Use the universal 1536 soybean linkage panel 1.0 for BSA and QTL mapping for new rust resistance genes**
 - Reduces cost of marker analysis and will have sufficient number of polymorphic markers for gene discovery
 - Maps populations in 3 days



Thanks

- Perry Cregan, USDA-ARS, Beltsville, MD
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