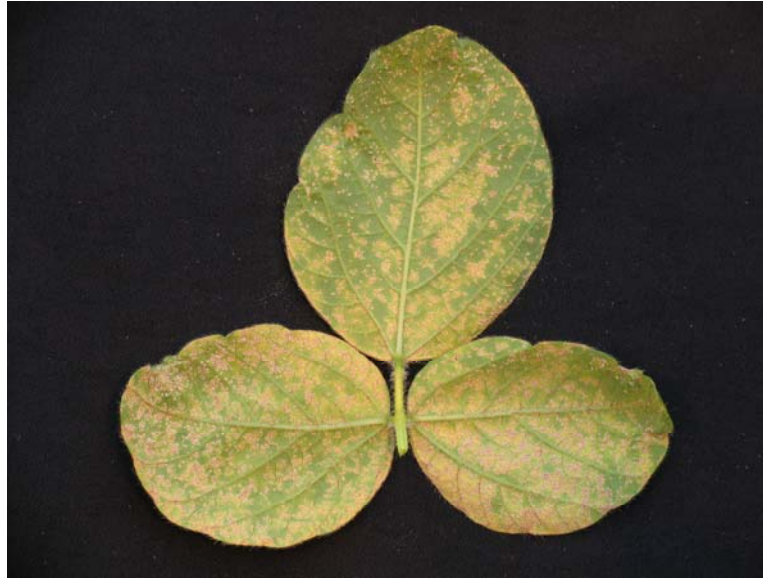


Phenotypic and Genetic Variation Among Soybean Rust Isolates



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***Phakopsora pachyrhizi* isolates in the US**

- How do the US isolates compare to isolates collected in other parts of the world?
- What is the origin of the isolates collected in the US?
- Are the US isolates genetically similar?
- How virulent are the US isolates?

Research Objectives

- Compare the level of virulence of each isolate using resistant and susceptible genotypes as standards
- Develop molecular markers for the known isolates of *P. pachyrhizi*

Reaction Phenotypes

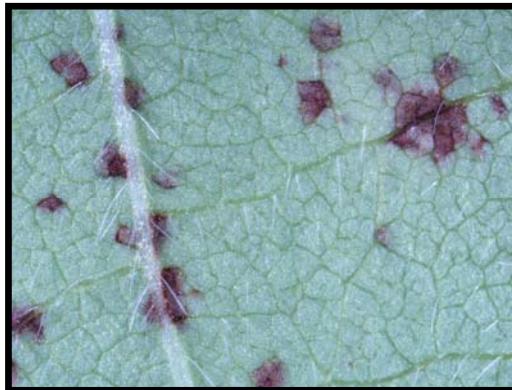
Immune



Red-brown



Tan



Resistance to *P. pachyrhizi*

- Varying degrees of resistance are conferred by the four resistance genes – *Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4*
- Resistance and susceptibility are dependent on the genotype of the host and the pathogen

	<i>Rpp1</i>	<i>Rpp2</i>	<i>none</i>
Isolate 1	Immune	RB	Tan
Isolate 2	RB	RB	Tan
Isolate 3	Tan	Tan	Tan



- Australia 79-1
- Brazil 01-1
- Brazil 01-2
- China 03-1
- Columbia 04-1
- Columbia 04-2
- Columbia 04-3
- India 73-1
- Paraguay 01-2
- Paraguay 01-3
- Paraguay 01-4
- Philippines 77-1
- South Africa 01-1
- Taiwan 72-1
- Taiwan 80-1
- Taiwan 80-2
- Thailand 01-1
- Thailand 02-1
- USA, Alabama 04-1
- USA, Alabama 04-2
- USA, Alabama 04-3
- USA, Hawaii 94-1
- USA, Hawaii 96-1
- USA, Louisiana 04-1
- USA, Louisiana 04-2
- USA, Louisiana 04-3
- Vietnam 05-1
- Zimbabwe 01-1

	<i>Rpp1</i>	<i>Rpp2</i>	<i>Rpp3</i>	<i>Rpp4</i>	<i>Rpp ?</i>	none
Australia 79-1	Immune	Red-brown	Red-brown	Red-brown	Red-brown	Tan
Brazil 01-1	Tan	Red-brown	Tan / Red-brown	Red-brown	Tan / Red-brown	Tan
Brazil 01-2	Tan	Red-brown	Tan / Red-brown	Red-brown	Tan / Red-brown	Tan
China 03-1	Tan	Red-brown	Tan	Red-brown	Tan	Tan
Columbia 04-1	Tan	Red-brown	Tan	Red-brown	Tan	Tan
Columbia 04-2	Tan	Red-brown	Tan	Red-brown	Tan	Tan
Columbia 04-3	Tan	Red-brown	Tan	Red-brown	Tan	Tan
India 73-1	Immune	Red-brown	Red-brown	Red-brown	Immune	Tan
Paraguay 01-2	Tan	Red-brown	Red-brown	Red-brown	Tan / Red-brown	Tan
Paraguay 01-3	Tan	Red-brown	Red-brown	Red-brown	Red-brown	Tan
Paraguay 01-4	Tan	Red-brown	Tan	Red-brown	Tan	Tan
Philippines 77-1	Tan	Red-brown	Tan	Red-brown	Tan	Tan
South Africa 01-1	Tan	Red-brown	Red-brown	Red-brown	Red-brown	Tan
Taiwan 72-1	Tan	Red-brown	Tan	Red-brown	Tan	Tan
Taiwan 80-1	Tan	Tan / Red-brown	Tan	Red-brown	Tan	Tan
Taiwan 80-2	Tan	Tan	Tan	Red-brown	Tan	Tan
Thailand 01-1	Tan	Red-brown	Tan	Red-brown	Tan	Tan
Thailand 02-1	Tan	Red-brown	Tan	Red-brown	Tan	Tan
● USA, Alabama 04-1	Tan	Red-brown	Red-brown	Red-brown	Red-brown	Tan
● USA, Alabama 04-2	Tan	Red-brown	Tan	Red-brown	Tan	Tan
● USA, Alabama 04-3	Tan	Red-brown	Tan	Red-brown	Tan	Tan
● USA, Hawaii 94-1	Immune	Red-brown	Red-brown	Red-brown	Immune	Tan
● USA, Hawaii 96-1	Immune	Red-brown	Red-brown	Red-brown	Immune	Tan
● USA, Louisiana 04-1	Immune	Red-brown	Red-brown	Red-brown	Immune	Tan
● USA, Louisiana 04-2	Tan	Red-brown	Tan	Red-brown	Tan	Tan
● USA, Louisiana 04-3	Red-brown	Red-brown	Red-brown	Red-brown	Red-brown	Tan
Vietnam 05-1	Tan	Red-brown	Tan	Red-brown	Tan	Tan
Zimbabwe 01-1	Tan	Red-brown	Tan	Red-brown	Tan	Tan

Research Objectives

- Compare the level of virulence of each isolate using resistant and susceptible genotypes as standards

- Develop molecular markers for the known isolates of *P. pachyrhizi*

- Determine genetic similarity among the isolates
- Reliable identification of each isolate
- Population genetic studies that monitor gene flow

Molecular Markers for Differentiating Soybean Rust Isolates

- Simple Sequence Repeats (SSRs) / Microsatellites
 - Tandem repetitions of a few base pairs
 - Highly abundant in Eukaryotic genomes
 - SSRs exhibit extensive levels of polymorphisms
 - Sequence flanking SSRs are conserved

Microsatellites – Simple Sequence Repeats (SSR)

Locus X



Allele 1



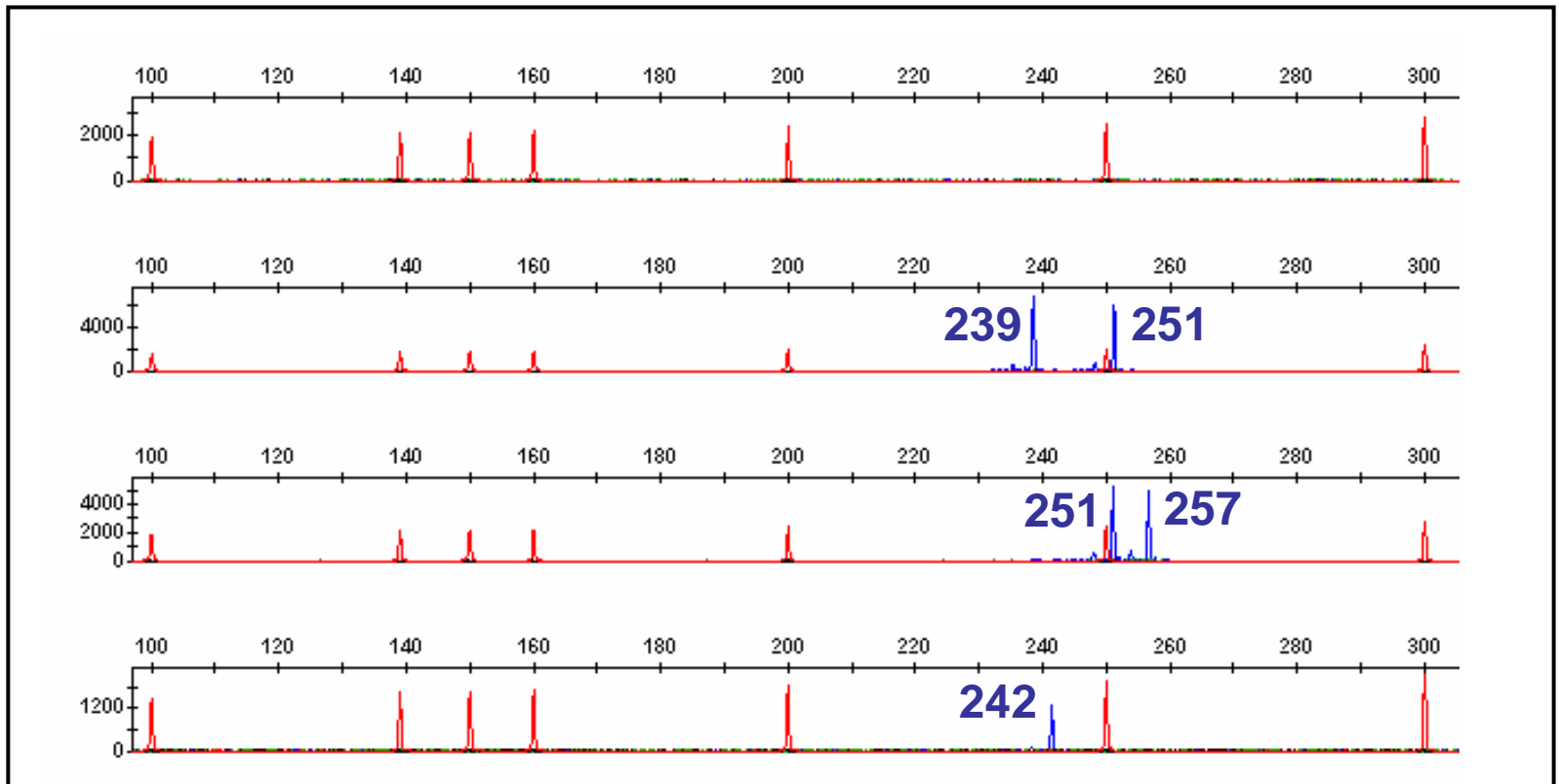
Allele 2



Allele 3

Microsatellite Analysis

- Products separated and detected on an ABI 3130xl
- “Peak calls” made with ABI GeneMapper v3.7 software



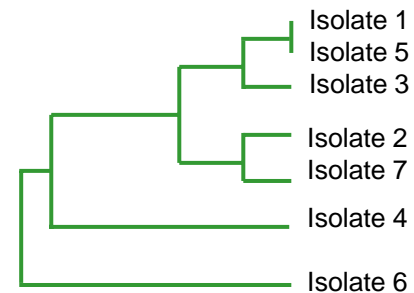
Data Analysis

Marker 1

Marker 2

	allele 1	allele 2	allele 3	allele 4	allele 5	allele 1	allele 2	allele 3
Isolate 1	+	-	-	+	-	+	+	-
Isolate 2	+	-	+	+	+	+	-	+
Isolate 3	+	-	+	+	+	+	+	+
Isolate 4	+	+	+	-	-	-	+	+
Isolate 5	+	-	-	+	-	+	+	-
Isolate 6	-	+	+	-	-	-	+	+
Isolate 7	+	-	+	+	-	+	-	+

- PCR Products scored as present (+) or absent (-)
- Jaccard similarity coefficients calculated for all isolate pair combinations to produce a similarity matrix
- Similarity matrix submitted for UPGMA cluster analysis and the Principle Coordinates ordination analysis (NTSYS)

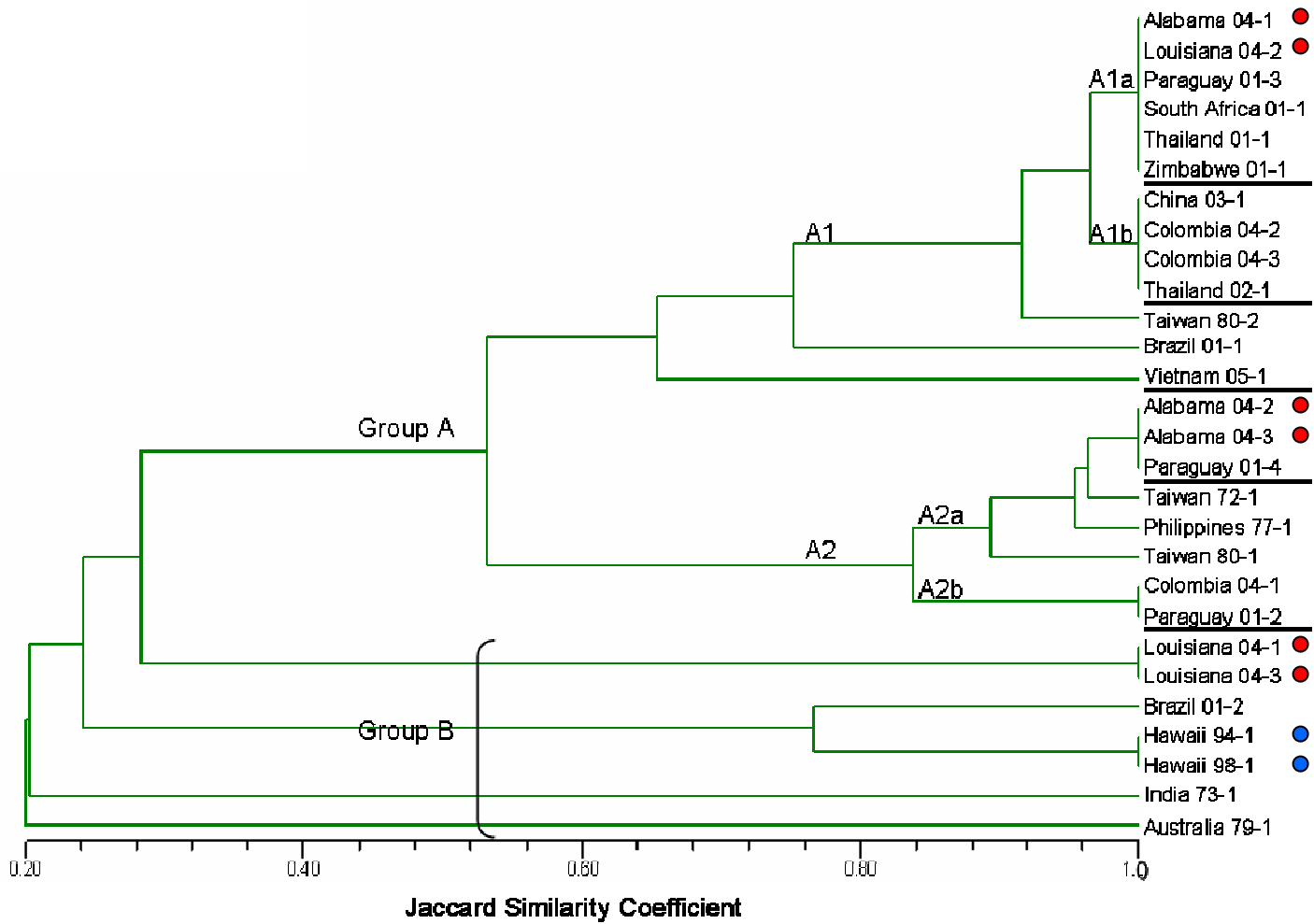


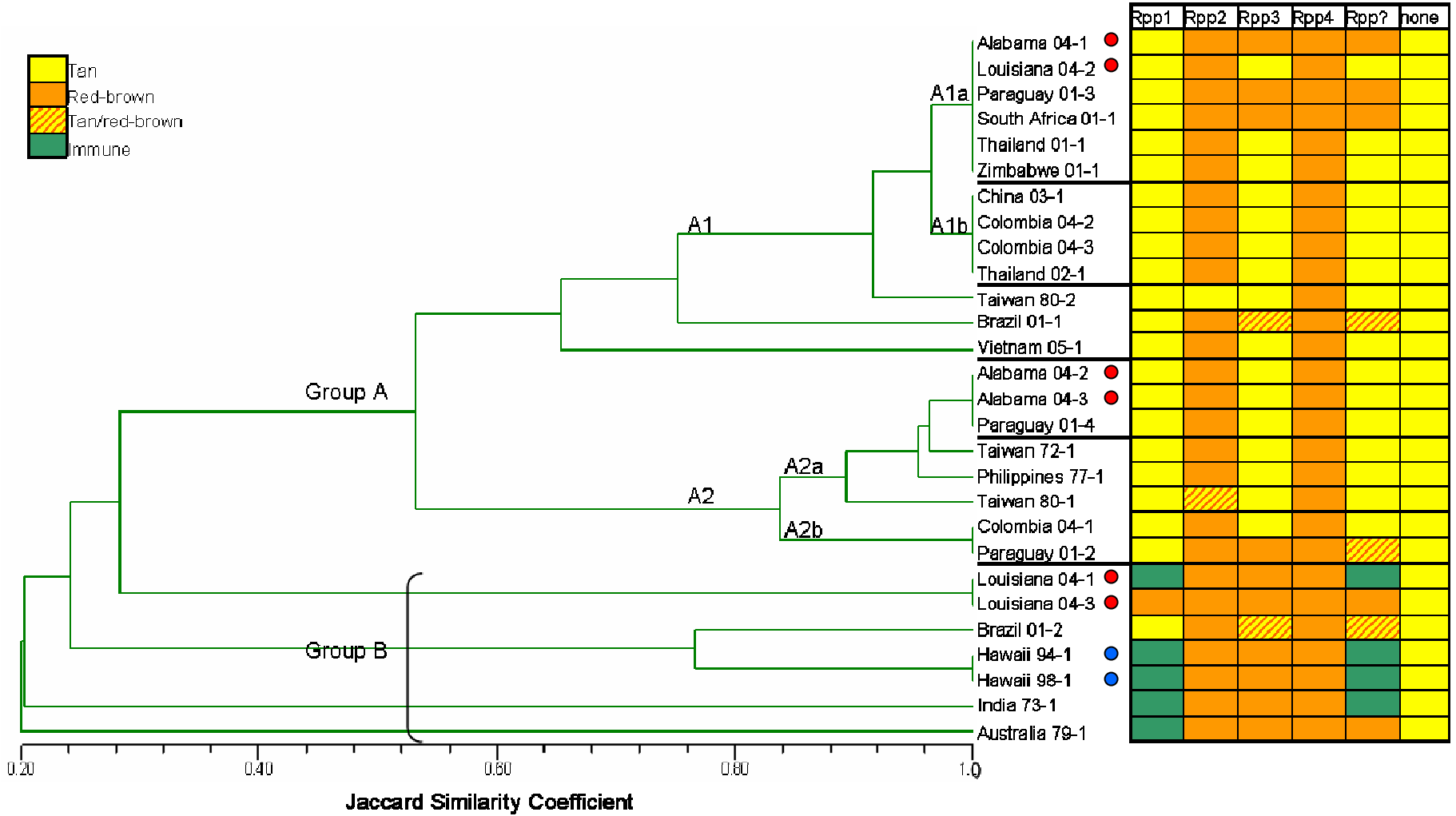
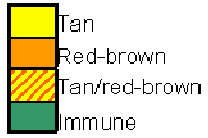
Phakopsora pachyrhizi SSRs

- Repeatmasker and StrFinder programs were used to mine microsatellites from *P. pachyrhizi* genomic sequences deposited in GenBank
- 1860 unique SSR sites were found within the nuclear genome
- 82 markers were evaluated using TW72-1 genomic DNA as the template
- 24 SSRs were selected and used to analyze select soybean rust isolates

Microsatellites used for the analysis

<u>Marker</u>	<u>Motif</u>	<u># of alleles</u>	
PpSSR1	AGA	5	
PpSSR2	TGT	7	
PpSSR3	TC	6	
PpSSR4	AT	2	
PpSSR5	CT	7	
PpSSR6	TC	3	
PpSSR7	TGA	5	
PpSSR8	TA	3	
PpSSR9	AT	2	invariant
PpSSR10	AT	1	invariant
PpSSR11	AT	4	
PpSSR12	TC	4	
PpSSR13	AT	3	
PpSSR14	AAT	5	
PpSSR15	AG	2	
PpSSR16	AT	4	
PpSSR17	TTA	3	
PpSSR18	GAA	1	invariant
PpSSR19	TA	5	
PpSSR20	CAAT...TTA	3	
PpSSR21	TA	3	
PpSSR22	TA	4	
PpSSR23	TCA	8	
PpSSR24	AT	3	





Conclusions

- Nineteen of the SSR markers were informative for assessing genetic variation among the isolates
- There is much genetic diversity with the FDWSRU collection
- Analysis of the SSR data reveals two distinct clusters (designated Group A and Group B)
- Groups A and B also show distinct reaction phenotypes when tested on different soybean accessions
- The six isolates collected from Louisiana and Alabama in November 2004 are genetically diverse, and show different reaction phenotypes on soybean accessions containing *Rpp1-4*

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