Genetic Resistance of Soybean to Frogeye Leaf Spot, Mapping of Rcs3 Gene, and Breeding for Resistance

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Acknowledgements: Drs. R. Boerma, D. Phillips, Ali Missaoui, David Walker, J. Bond, G. Shannon, A. Wrather, B.K. Ha, M. Newman, and A. Mengistu

Overview

- History of the disease
- Frogeye leaf spot (FLS) symptoms and damage
- Genetic resistance of soybean to FLS
- Molecular mapping and markers for Rcs3 gene
- Breeding for FLS
- Summary

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History

- FLS is a common disease of soybean in most soybean growing countries in the world
- First reported in Japan in 1915 and in the USA in 1924
- Common problem in the southern soybean region in USA
- Recently has become a problem in many northern soybean states (OH, IN, IL, MO, IA)

The pathogen and Disease Development

- Foliar disease caused by a fungus, *Cercospora sojina* K. Hara previously known as *Cercospora daizu*
- Inocula -spores (conidia) which are produced on infected leaves and residues of leaf, stem and infested seed
- Spores are readily spread short distances by wind and rain
- Young expanding leaves are highly susceptible, while fully expanded leaves are more resistant to invasion.

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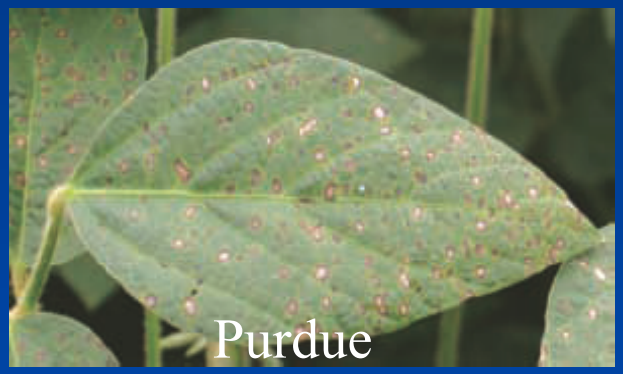
The pathogen and Disease Development

- Spore development and infection require warm and humid conditions
- Under favorable environment, disease cycles can continue from vegetative to late reproductive stages even infecting pods and seeds
- Uninfected seeds can be contaminated with spores or mycelium during harvest

Disease Symptoms on leaves

- Lesions are circular to angular spots
- Lesions initially appear as dark water soaked spots that sometimes have lighter centers
- Lesions develop into brown spots surrounded by narrow dark reddish brown margins
- Eventually adjacent lesions may coalesce to form larger irregular spots



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Disease Symptoms-stem, pod and seed

- Stem- long narrow lesions can cover up to half of the stem surfaces
- Pod- lesions are circular to elongate and slightly sunken, with a reddish brown color
- Seeds - conspicuous light to dark gray or brown areas that can range from specks to large blotches

http://www.ces.purdue.edu/extmedia/BP/BP-131-W.

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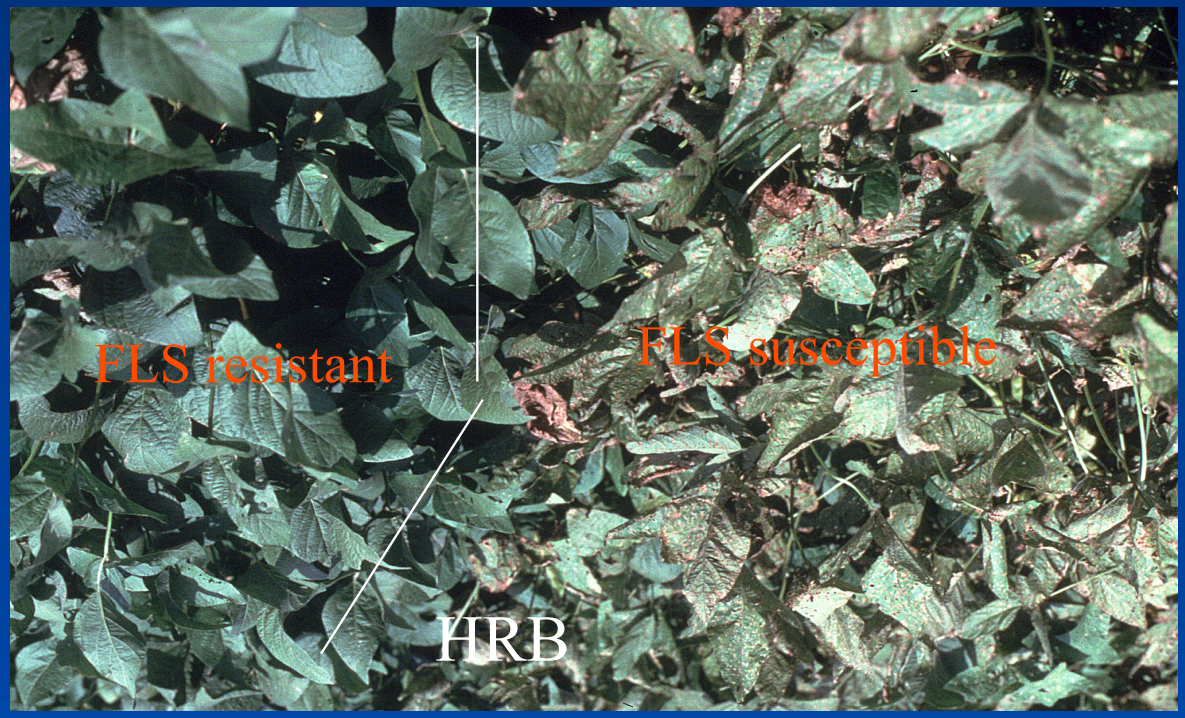
Seed Yield Loss from FLS

- Yield loss from FLS is mainly due to reduction in photosynthetic leaf area by lesions and/or premature defoliation

- Yield reductions from 10 to 60% due to FLS have been reported



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Estimated Seed Yield Loss from FLS

	Seed yield loss in thousand bushels			
Region	2006	2007	2008	Total
Southern	2966(6)	750(8)	2410(5)	6,126
Northern	9716(9)	8695(9)	4346(15)	22,757
Total	12,682	9,445	6,756	28,883

In a list of 24, FLS ranks between 5-8th in south and 9-15th in North.
FLS is not high on the list, but it impacts soybean in both south and north

Data Source: Dr. Allen Wrather, Univ. of Missouri Delta Center

How to manage FLS?

- Genetic resistance is the best option
- Use clean pathogen free seeds and treat seeds with fungicide if possible
- Deep plough soybean residues (contrary to no-till) into the soil and use a 2-yr rotation with non-host crops
- Use fungicides at the late flowering and beginning seed (R2-R5) growth stages if needed

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Genetic resistance to FLS

- Univ. of Georgia has >90 isolates (races??) of *C. sojina*
- *Rcs1* from `Lincoln' for resistance to race 1&5 (Athow and Probst 1952) and *Rcs2* from `Kent' for resistance to race 2 (Athow et al. 1962)

- *Rcs3* from 'Davis' provides resistance to all races (Boerma and Phillips 1983)

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Races of *C. sojina* and soybean differentials

- FLS research has been complicated due to 38 differentials and large number (??) of races
- 10 differentials and representative 13 races were proposed (R. Mian, A. Missaoui, D. Walker, D. Phillips, and R. Boerma. 2008. Crop Sci.)

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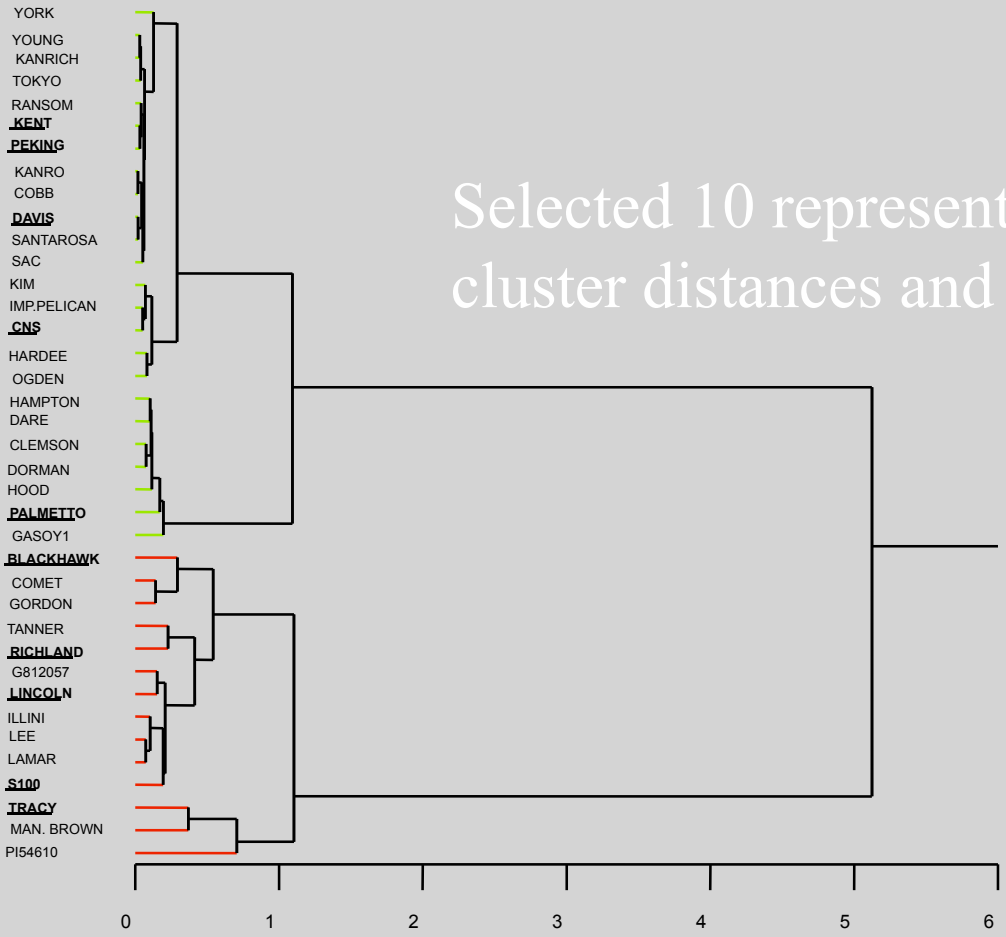
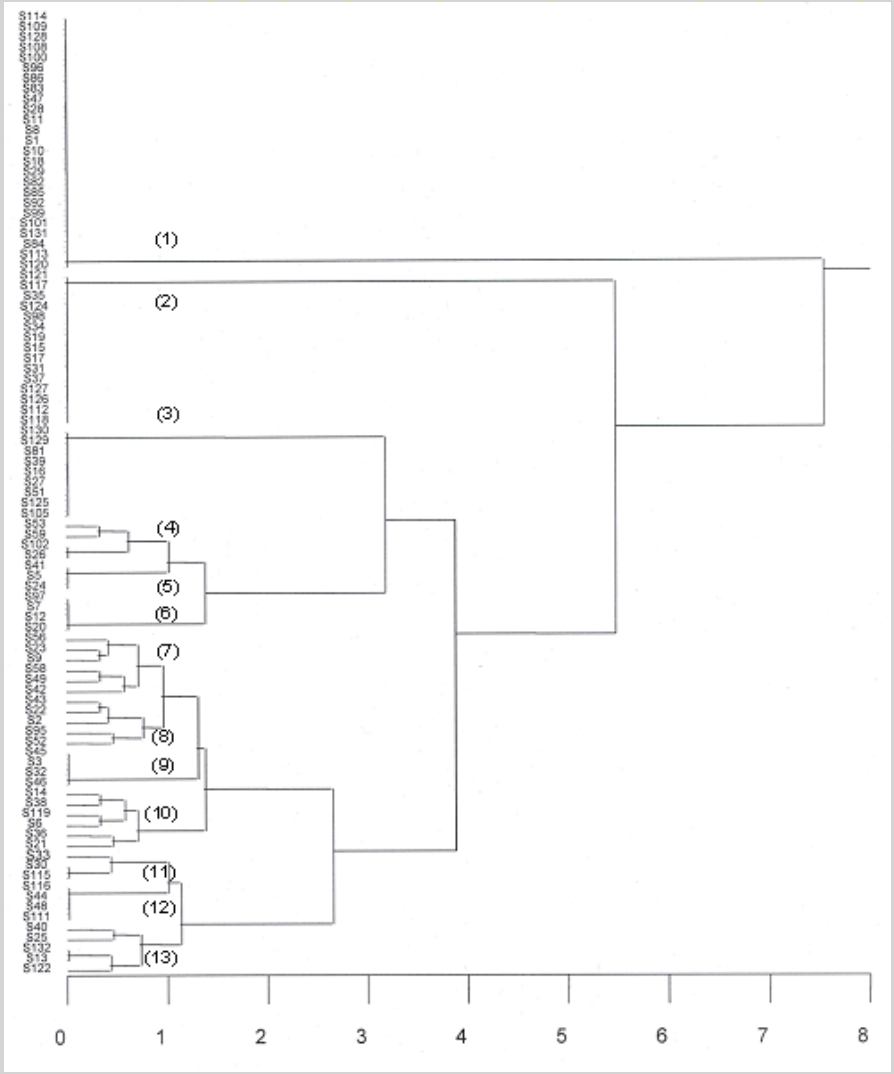
Clustering of 38 Soybean Differentials based on Reaction to 93 Isolates of *C. sojina*



Selected 10 representative differentials – cluster distances and pedigree

Mian et al. 2008. Crop Sci.

Cluster of 93 isolates of *C.sojina* based on the Reaction of 10 Differentials



Mian et al. 2008. Crop Sci.

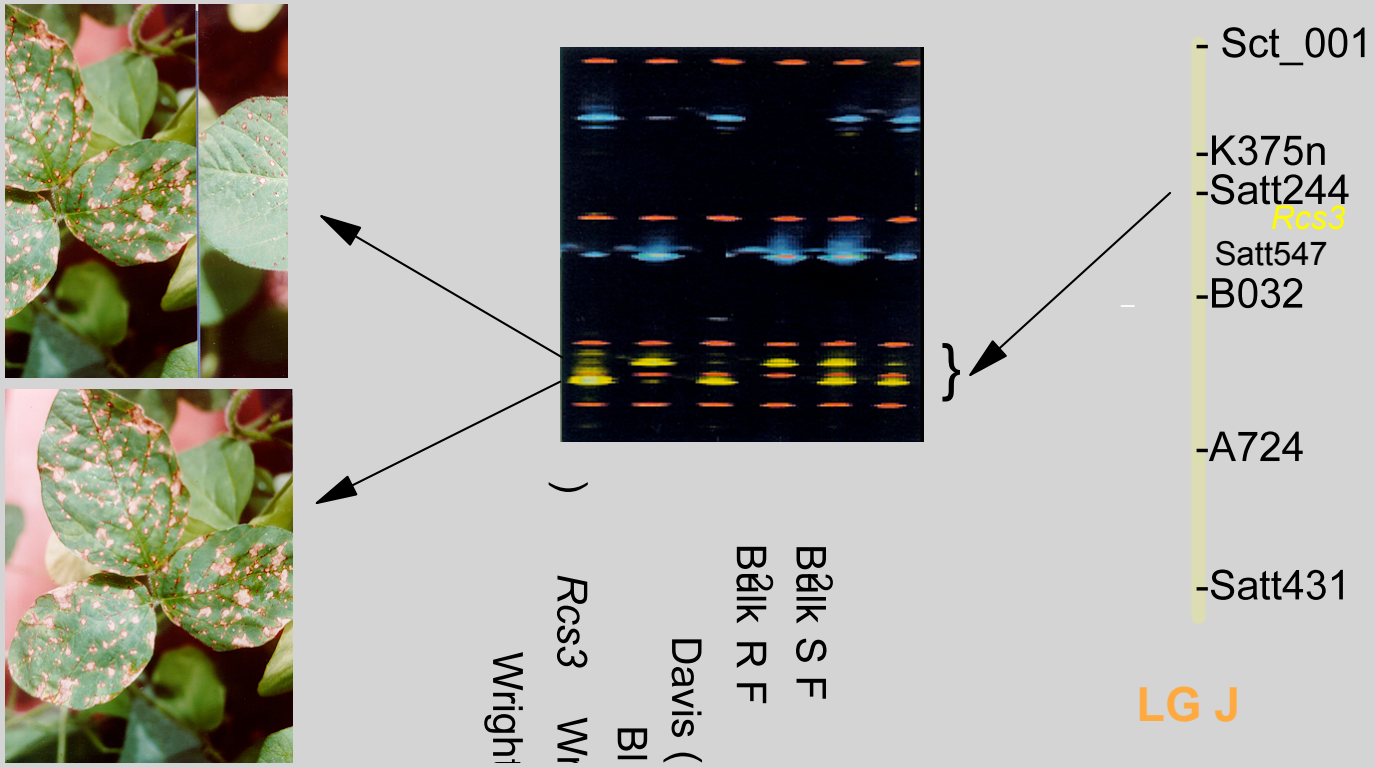
Proposed Classification of *C. sojina* Isolates

	Race designation/ (isolate)												
Differential	R5 (S5)	R6 (S12)	R7 (S95)	R8 (S102)	R9 (S9)	R10 (S125)	R11 (S1)	R12 (S32)	R13 (S25)	R14 (S127)	R15 (S33)	R16 (S116)	R17 (S21)
Davis	-	-	-	-	-	-	-	-	-	-	-	-	-
Peking	-	-	-	-	-	-	-	-	+	-	-	-	+
Kent	-	-	-	-	-	-	-	-	-	-	+	-	+
CNS	-	-	+	-	-	-	-	-	-	-	+	+	+
Palmetto	-	-	-	-	+	-	-	+	-	+	+	+	+
Tracy	-	-	-	-	-	-	+	-	+	+	+	+	-
Lincoln	-	-	-	+	+	+	+	+	+	+	-	+	+
S100	-	-	+	-	-	+	+	+	+	+	+	+	+
Richland	+	-	-	+	+	+	+	+	+	+	+	+	+
Blackhawk	+	+	+	+	+	+	+	+	+	+	+	+	+

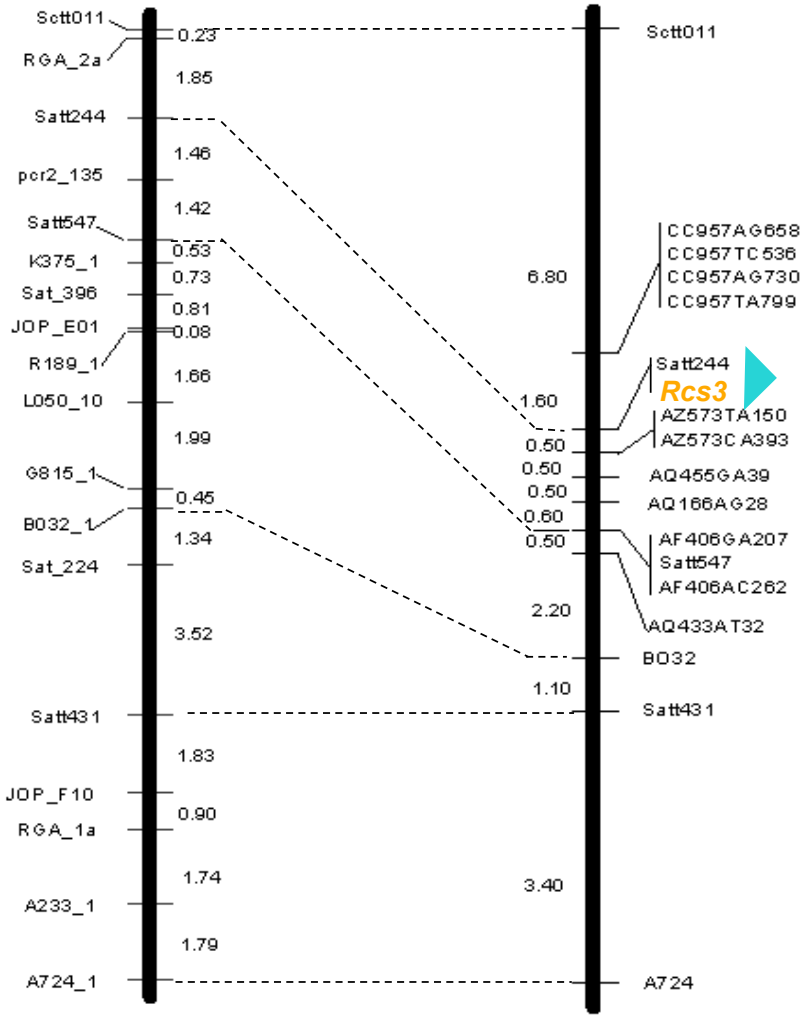
+ indicates compatible reaction (sensitive)
- Indicates incompatible reaction (resistant).

Mian et al. 2008. Crop Sci.

Genetic Mapping (BSA) of Rcs3



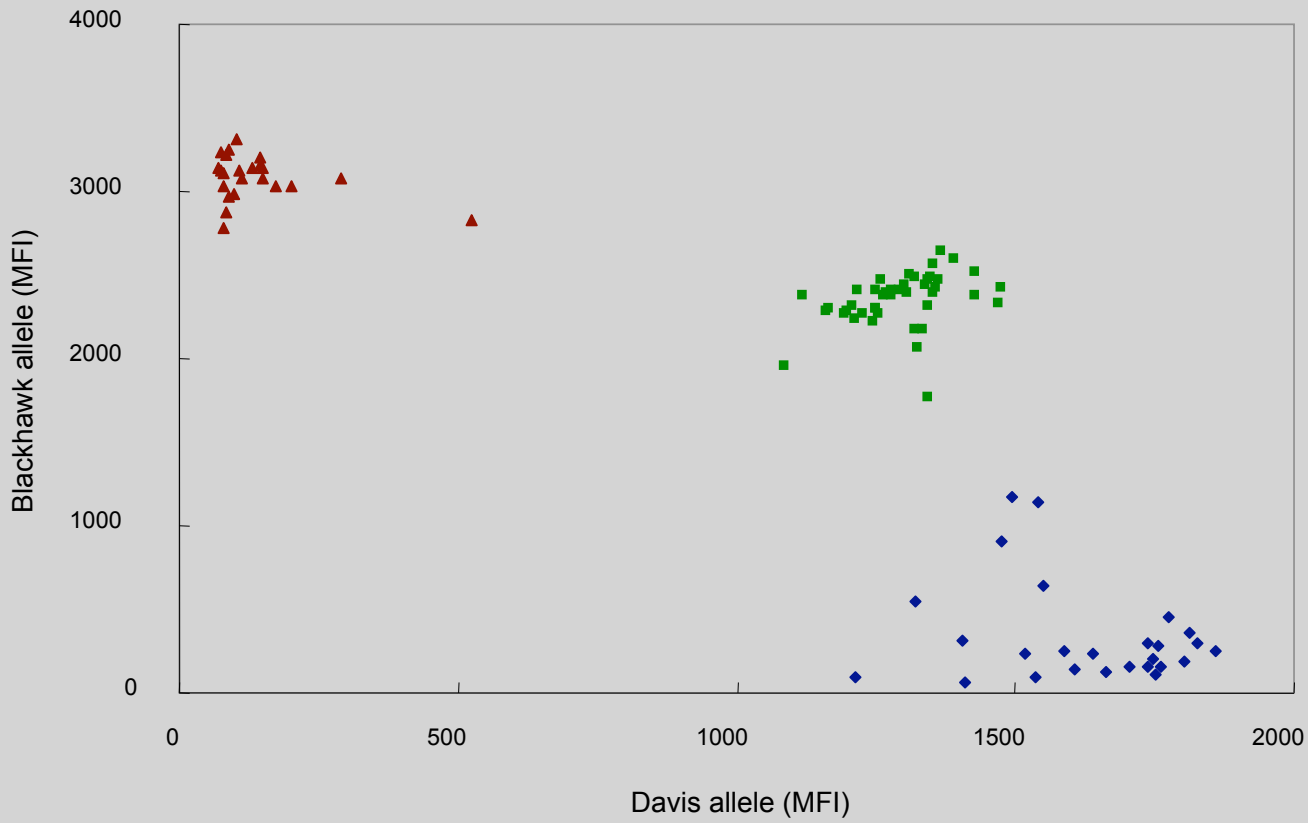
Mian et al. (1999). Crop Sci. 39:1687-1691



Missaoui et al. 2007. Crop Sci.

- SNP markers - just AZ573TA150/CA393 or several SNPs (multiplexed) for MAS
- Low cost (DH) assay has been developed for MAS and it works very well

Direct Hybridization Assay for MAS



MAS for Rcs3 among 65 Northern maturity (II-V) FLS resistant soybean cultivars/breeding lines

	Markers				
Genotype	AQ166AG280	AQ455GA396	AZ573TA150	Satt-244	CC957AG730
Davis*	GG	GG	DD	DD	AA
Wright-*Rcs3* *	GG	GG	DD	DD	AA
Progeny 5715 RR	GG	GG	DD	DD	AA
Progeny 5770	GG	GG	DD	DD	AA
Dpl 5915	GG	GG	DD	DD	AA
Gateway 4R485	GG	GG	DD	DD	AA
Pioneer 94B73	GG	GG	DD	DD	AA
Pioneer 94M41	GG	GG	DD	DD	AA
LN97-15076	GG	GG	DD	DD	AA
Delta Pine 5414RR	GG	GG	DD	DD	AA
FFR 5663	GG	GG	DD	DD	AA
Hornbeck R5624	GG	GG	DD	DD	AA
Delta King XT J554	GG	GG	DD	DD	AA
Progeny 4949 RR	GG	GG	DD	DD	AA
S99-2281	GG	GG	DD	DD	*GG*
Lee**	*AA*	*AA*	*BB*	*BB*	*GG*
Blackhawk**	*AA*	*AA*	*BB*	*BB*	*GG*
Wright**	*AA*	*AA*	*BB*	*BB*	*GG*

Mian et al. 2009. Plant Dis.

MAS for Rcs3 among 64 (II-V) FLS resistant plant introductions

ID	LC573	CC957AG730	CC957AG7302	AQ166AG280	AQ166AG2802	AQ455GA396	AQ455GA3962
Davis	+	985	38	33	57	435	69
Wright Rcs	+	1042	49	39	73	406	67
Blackhawk	-	195	395	53	66	119	290
PI 437726	+	1236	114	49	91	593	88
PI 438302B	+	933	56	47	62	408	74
PI 494851	+	772	54	51	82	422	83

Breeding for FLS Resistance (Rcs3 gene)

- Conventional approach: screening for resistance in greenhouses
- Marker assisted breeding (MAB)
- The best approach: MAB followed by greenhouse confirmation of advanced breeding lines

Screen with one *C. sojina* race at a time!!!

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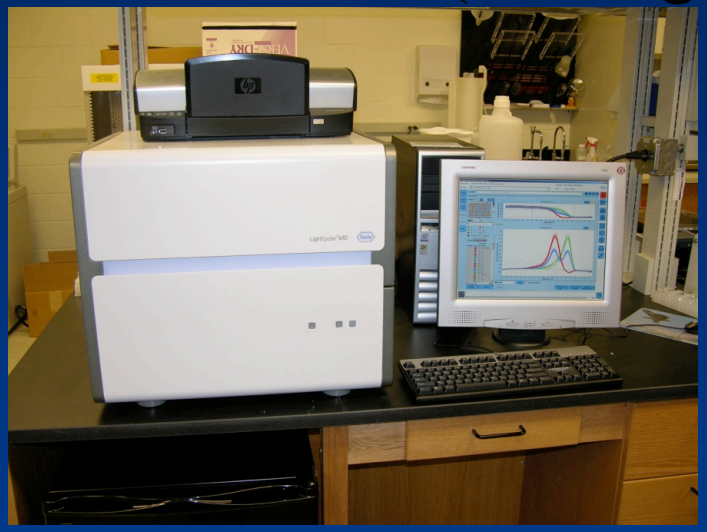
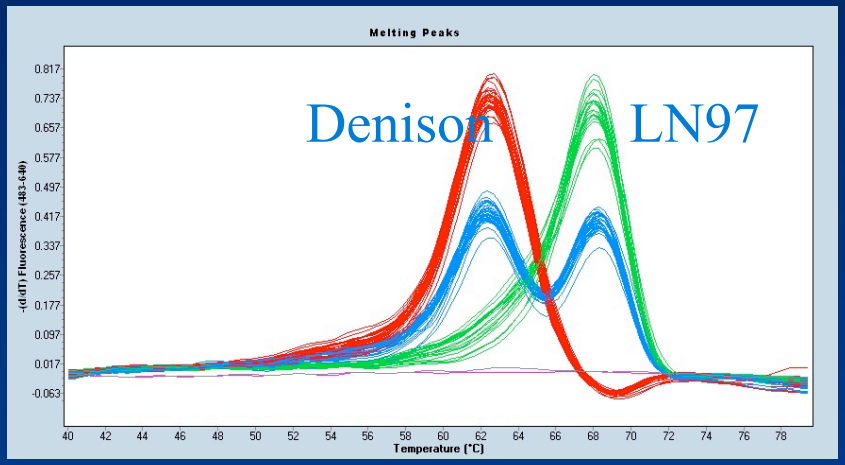
MAB for Rcs3 using LightCycler 480 Real-time PCR (melting curve analysis)





- Use a single probe developed at UGA (Ha and Boerma.2008. JCSB)
- 384 samples can be probed in 2 hours, much faster than Luminex.
- Homozygous BC2F2:3 with Rcs3 (Denison x LN97-10576; Wooster x LN97).
- Greenhouse confirmation underway (by Anne Dorrance)

Summary

- FLS reduces soybean yield in both north and south regions of USA
- Plant Pathologists and Breeders continue to identify new sources of FLS resistance
- Genetic characterizations (inheritance and mapping) of resistance genes in the new sources clearly lag behind
- The Rcs3 gene identified nearly 30 years ago still works against *C. sojina* races in USA, but it is time to identify and map new soybean genes for FLS resistance

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