



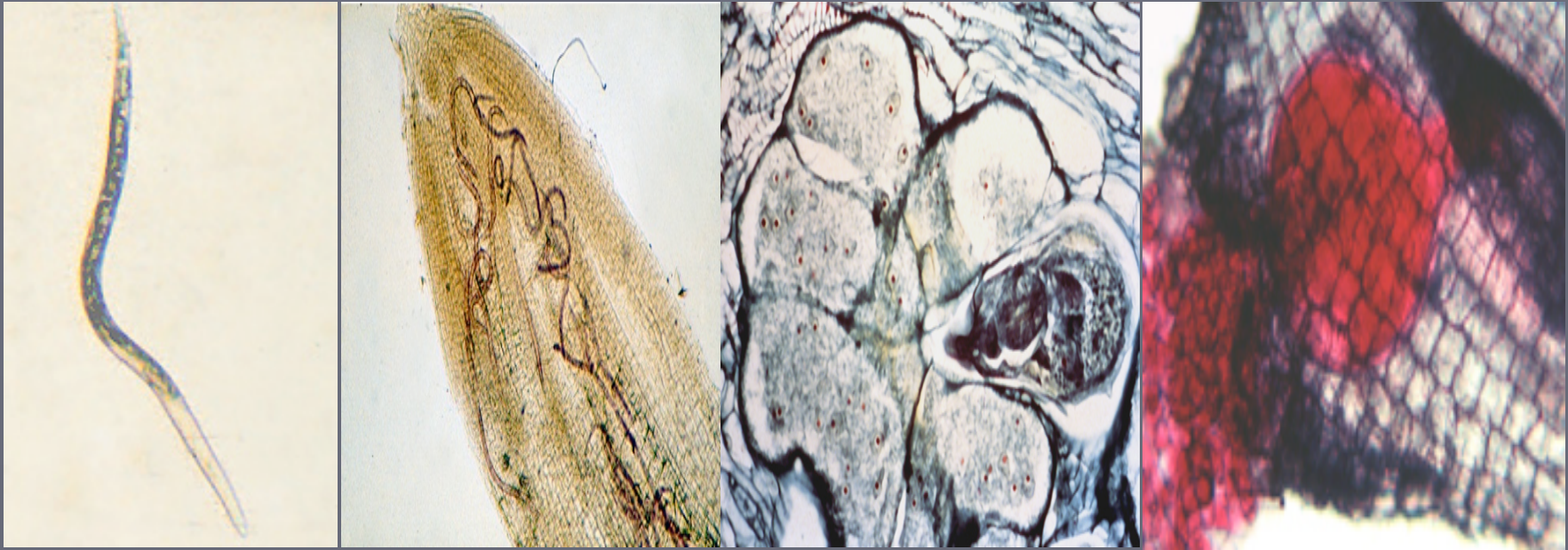
Breeding soybean for resistance to root-knot nematode

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The University of Georgia
The University of

Root-knot nematode



- *Meloidogyne* spp.
- Plant parasitic nematode (sedentary endo-parasitic)
- Wide host range (>2,000 plant species)

Soybean



- Southern root-knot (*M. incognita*) (Mi)
- Javanese root-knot (*M. javanica*) (Mj)
- Peanut root-knot (*M. arenaria*) (Ma)
- Yield losses in the United States were 9.6 million bushels in 2008



Riggs & Schmitt, 1987; Wrather & Koenning, 2008;

Contributed by Roger Boerma

Host status of common crops



Nematode	Soybean	Cotton	Peanut	Corn
Southern root-knot	Host	Host	Non-host	Host
Peanut root-knot	Host	Non-host	Host	Host
Javanese root-knot	Host	Non-host	Non-host	Host
Reniform	Host	Host	Non-host	Non-host
Soybean cyst	Host	Non-host	Non-host	Non-host

Phenotypic selection in greenhouse



Contributed by Roger Boerma & Jennifer Yates



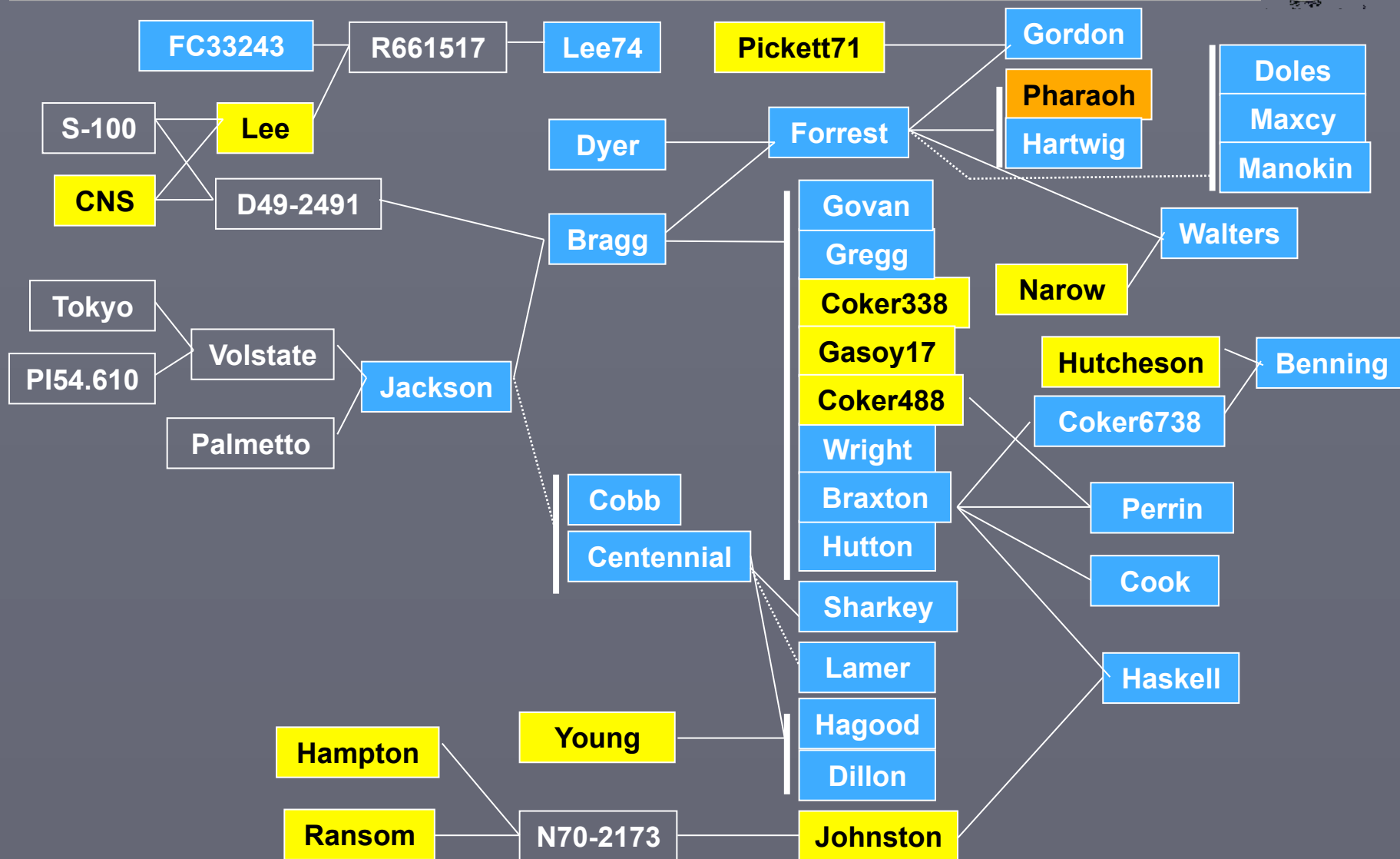
The Georgia Agricultural Experiment Stations
College of Agricultural and Environmental Sciences
The University of Georgia

Annual Publication 103
December 2009

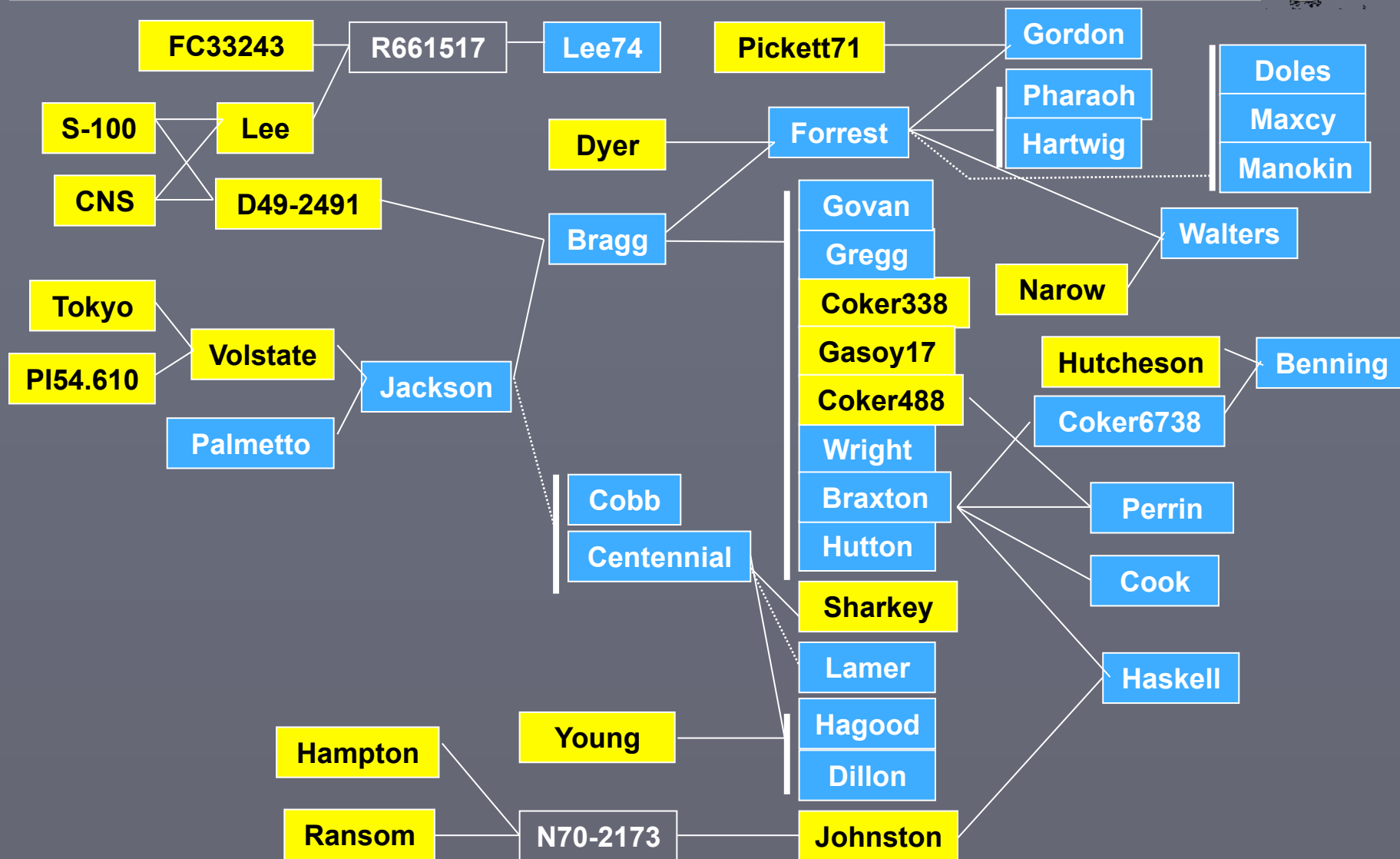
Greenhouse Ratings for Resistance to Three Species of Root-knot Nematode and Soybean Cyst Nematode, 2009

Company or Brand Name	Variety	Root-knot nematode			Cyst nematode	
		Southern ¹	Peanut ²	Javanese ³	Race 3 ⁴	Race 9 ⁵
		----- rating ⁶ -----			---- reaction ⁷ ----	
AgSouth	AGS 568 RR	2.0	4.5	5.0	R	R
AgSouth	AGS 606RR	4.5	5.0	4.5	R	S
AgSouth	AGS 747RR	1.5	4.8	3.0	R	S
AgSouth	AGS 758 RR	1.0	2.3	1.5	R	S
AgSouth	AGS Prichard RR	1.0	5.0	3.8	R	R
AgSouth	AGS Woodruff	2.8	4.8	4.0	R	S
AR	R01-2346	1.5	3.0	1.8	S	S
AR	R01-327	5.0	4.5	5.0	R	R
AR	R03-1232	5.0	2.5	3.0	S	S
AR	R04-357	5.0	5.0	3.5	S	S
Asgrow	AG5905	5.0	5.0	5.0	R	R
Asgrow	AG6301	1.0	5.0	5.0	S	S
Asgrow	AG6702	3.8	4.8	4.8	R	R
Asgrow	AG7501	2.8	5.0	4.8	R	S
Asgrow	AG7502	1.3	4.8	4.8	S	S
Asgrow	H7242 RR	1.3	3.8	2.0	R	S
Asgrow	DP5915RR	4.8	5.0	4.3	R	R
Asgrow	DP7330RR	1.0	4.0	4.3	S	S
Asgrow	DP7870RR	5.0	4.8	5.0	S	S
AU	Au02-2814	1.8	4.8	3.5	S	S
DynaGro	32B57	1.8	5.0	3.3	R	R
DynaGro	33C59	5.0	4.8	4.8	R	S
DynaGro	33X55	2.3	5.0	5.0	R	R
DynaGro	35F55	4.5	5.0	5.0	S	S
DynaGro	35K73	5.0	5.0	4.5	S	S

Pedigree and Mi reaction



Pedigree and Mi reaction



New sources of resistance



- Southern root-knot (Mi)

- **PI96354**

(Luzzi et al., 1987, Crop Sci. 27; Harris et al., 2003, Crop Sci. 43)

- **G93-9009**

(Luzzi et al., 1996, Crop Sci.36)

- Peanut root-knot (Ma)

- **PI200538**

- **PI594427C**

- **PI594651B**

(Luzzi et al., 1987, Crop Sci. 27; Harris et al., 2003, Crop Sci. 43; Yates et al., 2010, Crop Sci. 50)

- **G93-9106**

(Luzzi et al., 1996, Crop Sci.36)

- Javanese root-knot (Mj)

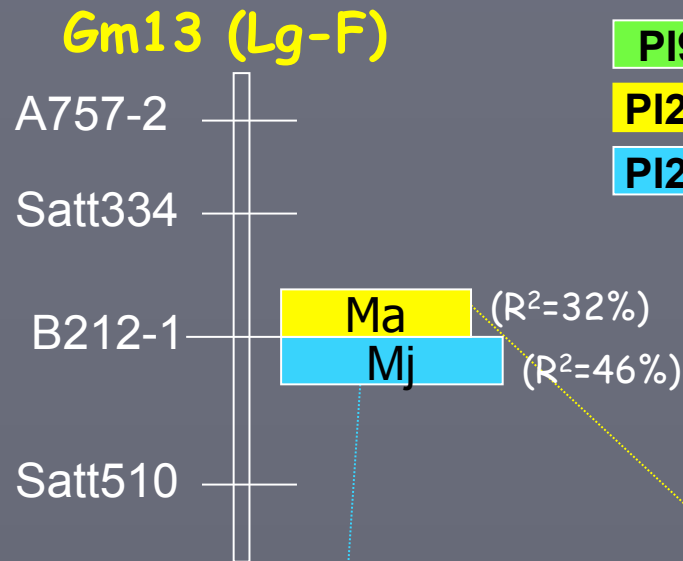
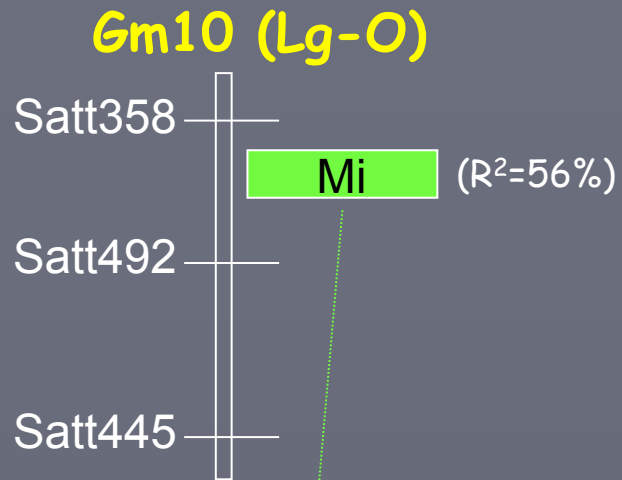
- **PI230977**

(Luzzi et al., 1987, Crop Sci. 27; Harris et al., 2003, Crop Sci. 43)

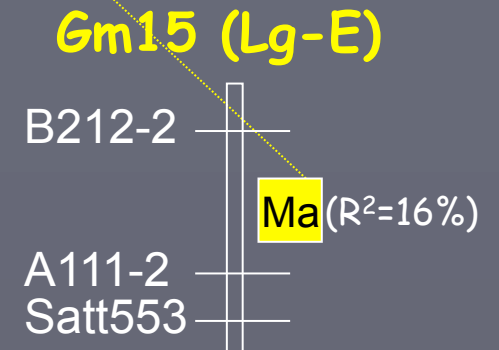
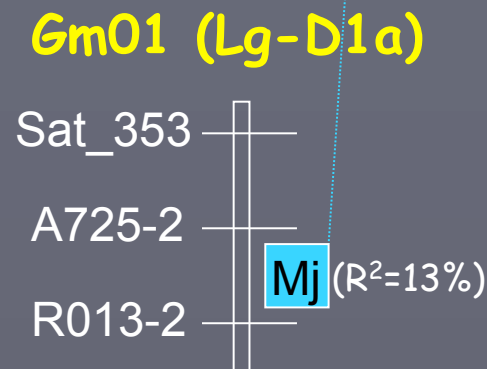
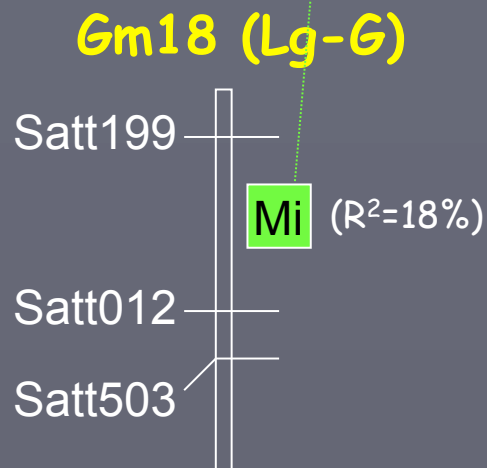
- **G93-9223**

(Luzzi et al., 1997, Crop Sci.37)

Resistance QTL for root-knot nematode



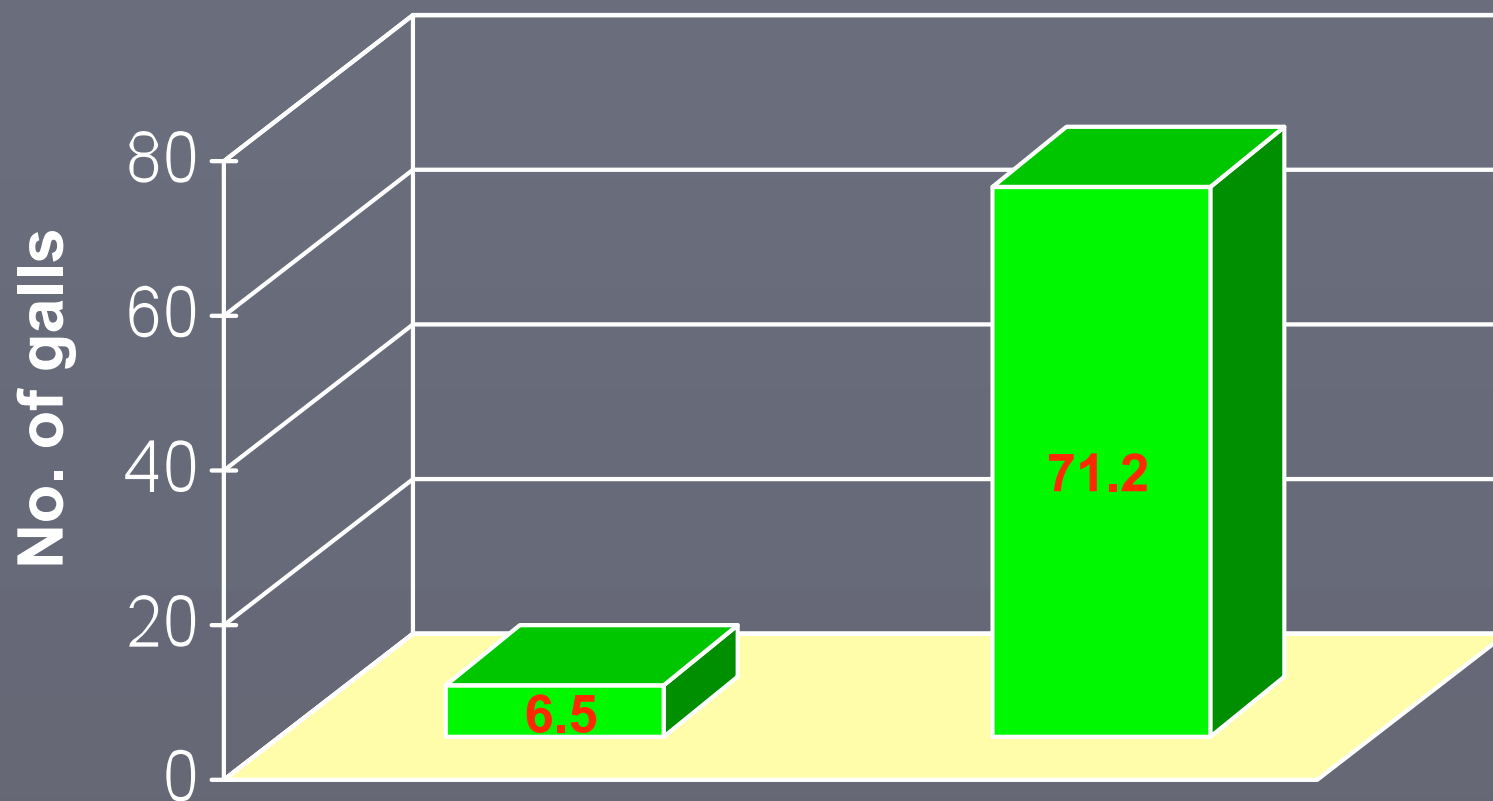
PI96354 Mi ($h^2=69\%$)
PI200538 Ma ($h^2=55\%$)
PI230977 Mj ($h^2=55\%$)



Effect of QTL for Mi



110 F₂ PI96354  Bossier



QTL on LG-O/G;

+/+

-/-

MAS for Mi QTL on Gm10 (Lg-O) using Satt358



92 F₆ Cook (R, 200bp)  N7001 (S, 192bp)

**Nematode
reaction**

Satt358
homoz. 200 bp

Satt358
homoz. 192 bp

Resistant lines

40

0

**Susceptible
lines**

1

51

SNPs identified from BAC



a) Satt358 containing genomic DNA clone on Gm10

```
PI96354 61 ATTATAAATGCTATCCTTTAATTCTTAGCTATGCGCTTTATGTAACAATACGATTTCTAT 12
0
BOSSIER 61 ATTATAAATGCTATCCTTTAATACTTAGCTGTGCGCTTTATGTAACAATACGATTTCTAT 12
0
*****
PI96354 151 TATTATTATTATTATTATTATTATTATTATTATTATTTTTTTCCTATTTTTTGGAAATATATT 21
0
BOSSIER 151 TATTATTATTATTATTATT-----ATTATATTTTTTGCCTATTTTTTGGAAATATATT 20
1
*****
```

b) Satt199 containing genomic DNA clone on Gm18

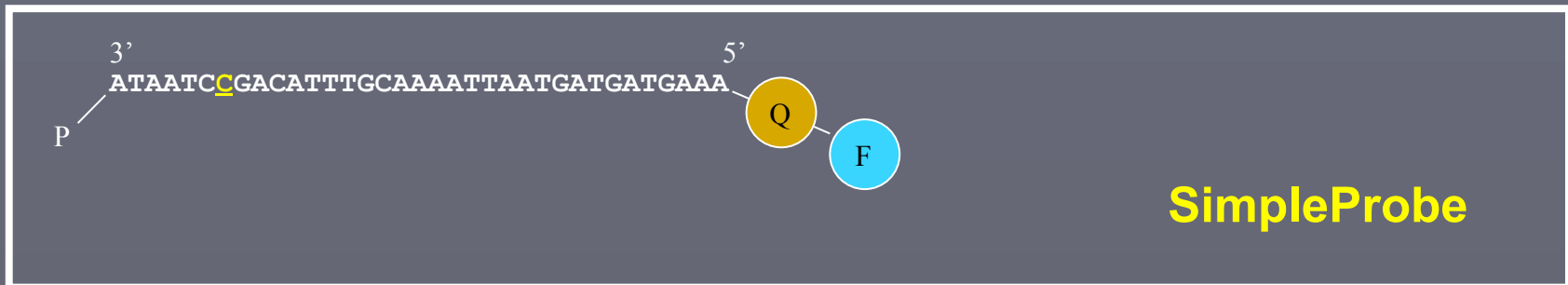
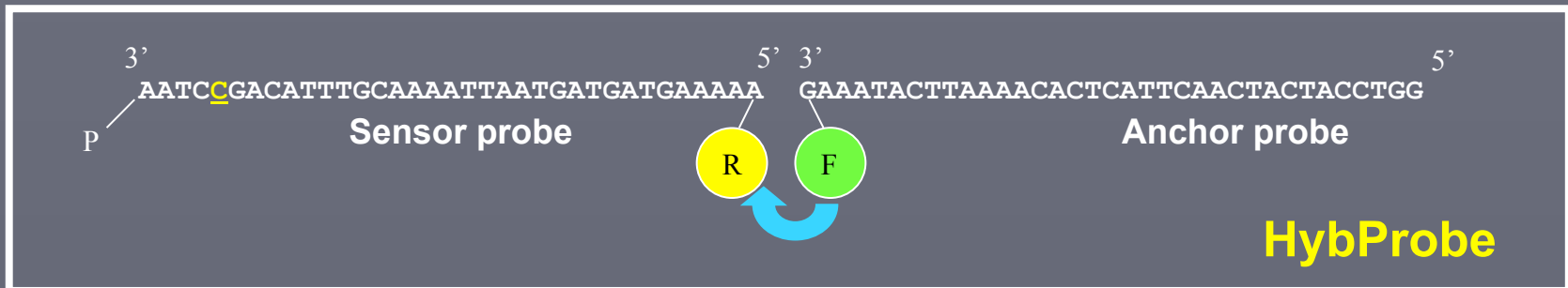
```
PI96354 61 TTTATATTTCTGTTTTCTCTTAA--AAAAAAAACCTAAAACAAAATGTCCACAGTAGTTG 12
0
BOSSIER 61 TTTATATTTCTGTTTTCTCTTAA--AAAAAAAACCTAAAACAAAATGTCCACAGTAGTTG 11
8
*****
PI96354 171 ATTATTATTATTATTATTATTATTATTATTATTAATATTAGACTGTAAACGTTTTAATTACTA 23
0
BOSSIER 169 ATTATTA-----TTATTATTATTATTATTAGGCTGTAAACGTTTTAATTACTA 21
6
*****
```

Melting curve analysis



SNP199 A/G

5' . /TATTAG_GCTGTAAACGTTTTAATTACTACTACTTTTTTACTTTTATGAATTTTGTGAGTAAGTTGATGATGGACC/ . 3'



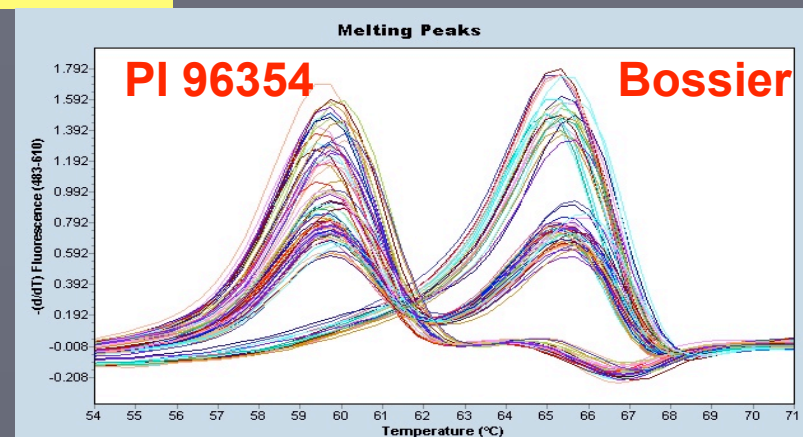
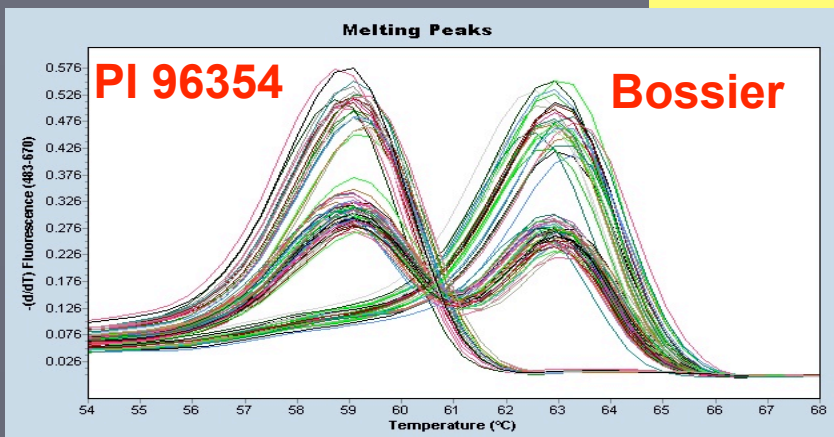
Two SNP genotyping assays



SNP199

HybProbe

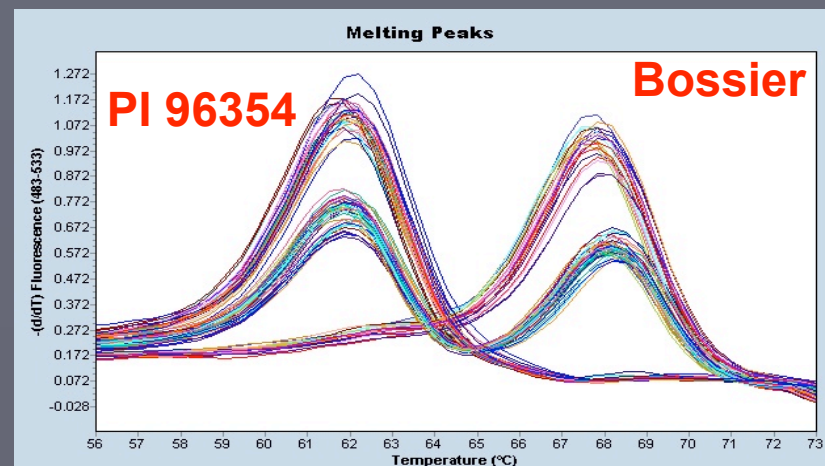
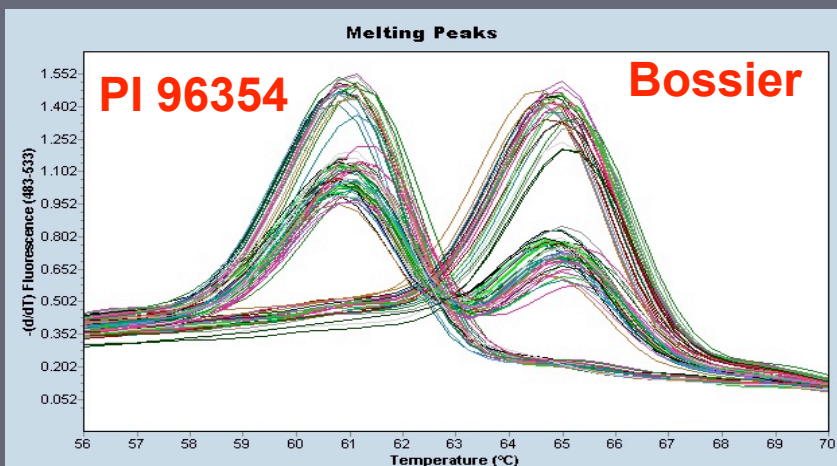
SNP358



SNP199

SimpleProbe

SNP358



Monsanto/UGA patent

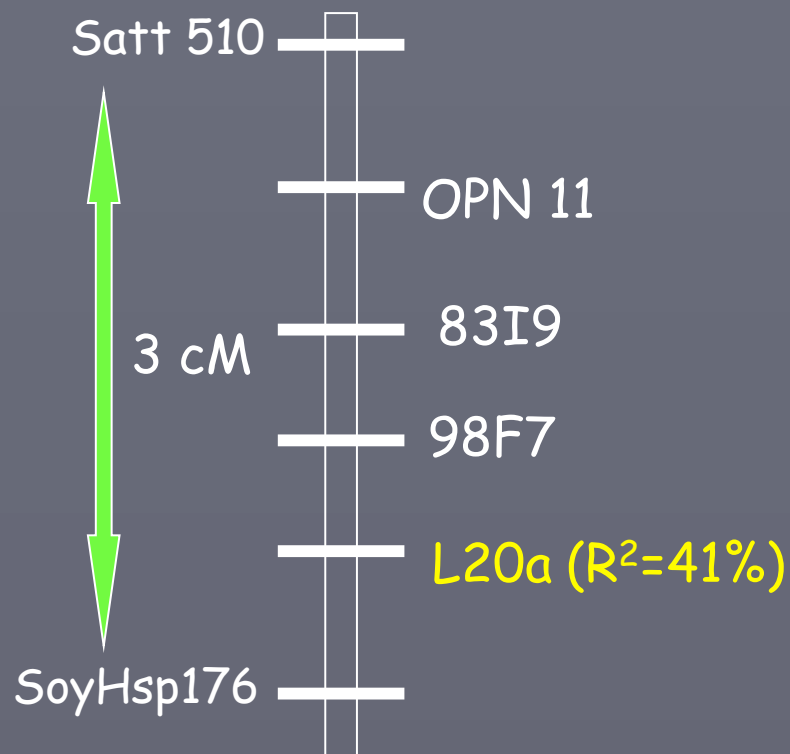


- US patent application No: 20090064354
 - Title : **Methods and compositions for selecting soybean plants resistant to southern root knot nematode**
 - Inventors: **Narvel, James; Concibido, Vergel; Cerny, Liesa; Tamulonis, John; Hancock, Floyd; Dougherty, Richard; Boerma, Henry Roger; Ha, Bo-Keun_**
 - Application Filed: Aug 6, 2008
 - Publication Date: Mar 5, 2009

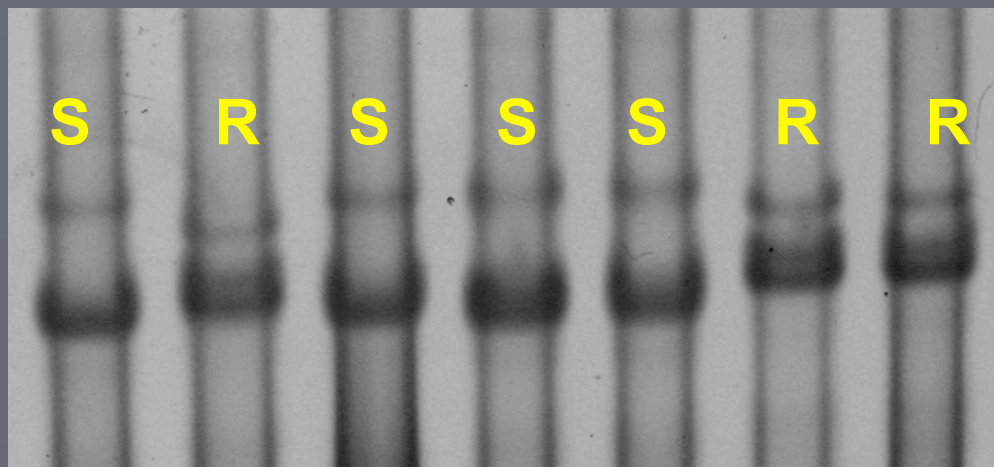
Fine mapping for Ma QTL



Gm13 (Lg-F)



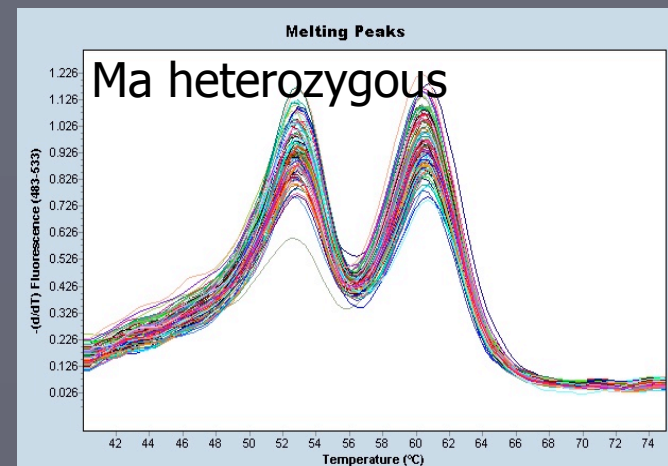
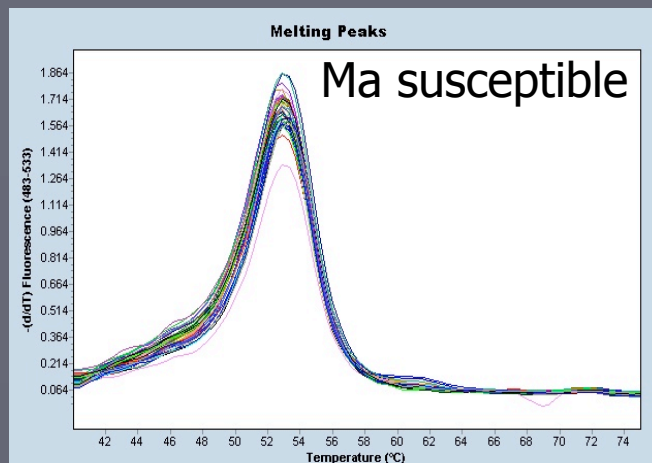
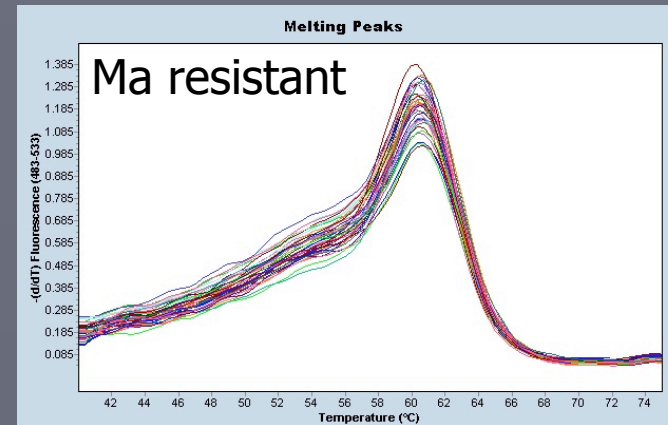
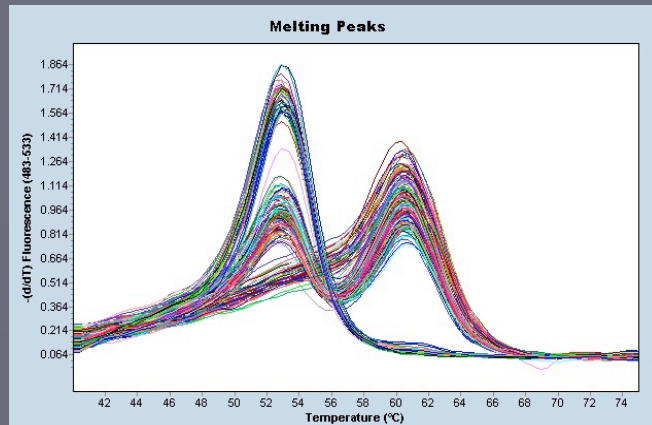
- PI200538
- PI594427C
- PI594651B
- PI230977 (Mj)



SNP genotyping assay for Ma



188 F₂ PI594427C x CNS



Time & cost



\$/data

Greenhouse screening

Planting + Inoculation + Gall counting = 35 days

\$ 5.00

LightCycler 480 (SNP)

DNA (1 week)

PCR setup

PCR/ melting curve

Genotype calling

\$ 0.23 ~ 0.27

ABI3730 (SSR)

DNA (1 week)

PCR setup

PCR

ABI3730

\$ 0.35 ~ 0.42

Time(hours)

1

2

3

4

5

New Mi QTL near the T-locus



Pubescence color
Gm06 (Lg-C2)
TT, or *Tt* = tawny
tt = gray

- Two backcross populations
 - BenningRR(5) x G93-9009
 - BoggsRR(6) x G93-9009
 - BenningRR and BoggsRR = tawny
 - G93-9009 = gray
- Phenotypic selection for high Mi resistance after each cycle of backcrossing
- Lines should have TAWNY
- Some of them were GRAY!

Greenhouse & field test



Lines	Entries (No.)	Mean (eggs plant ⁻¹)	Range (eggs plant ⁻¹)	Seed yield (kg ha ⁻¹)
Bossier	1	24920	n/a	
G93-9009	1	95	n/a	
BenningRR	6	225	85 - 393	3498a
BenningRR(5) x G93-9009				
Tawny lines	8	170a[†]	52.5 - 353	3128b[†]
Gray lines	6	42b	0 - 85	3302ab
Bossier	1	8080	n/a	
G93-9009	1	83	n/a	
BoggsRR	1	30	n/a	3101a
BoggsRR(6) x G93-9009				
Tawny lines	5	63a[‡]	6 - 111	3020a
Gray lines	5	6b	0 - 12	3107a

[†]LSD_(0.05)

[‡]LSD_(0.01)



Summary

- SNP assays for Mi resistance QTL on Lg-O and G
- SNP assays for Ma resistance QTL on Lg-F
- New confirmed sources of Ma (PI594427C & PI594651B)
- New QTL on Lg-C2 for Mi resistance
- SNP assays for reniform QTL (PI437564, unpublished)



Thanks to...

- **Bruce Luzzi** – Identification of new sources
- **Donna Harris** - Identification of new sources
- **John Tamulonis** – RFLP mapping for Mi, Ma, and Mj
- **Zenglu Li** – SSR mapping for Mi
- **Jennifer Yates** – Fine mapping for Ma
- **Zachary Shearin** – Mi QTL on Lg-C2



Thanks to...

- United Soybean Board
- Georgia Agricultural Experiment Stations
- Monsanto Company
- NSF Fellowship