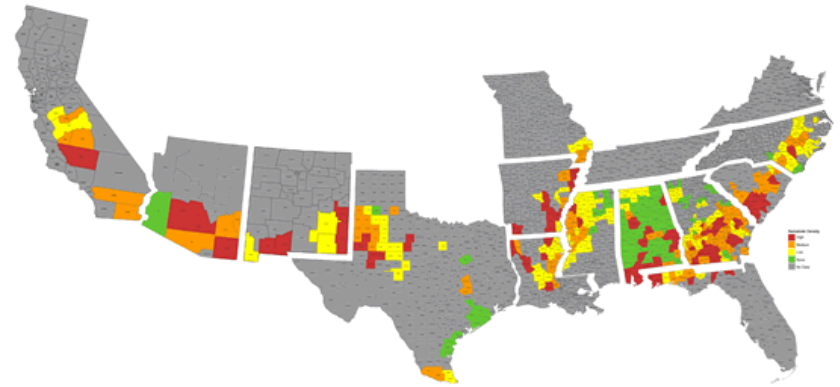


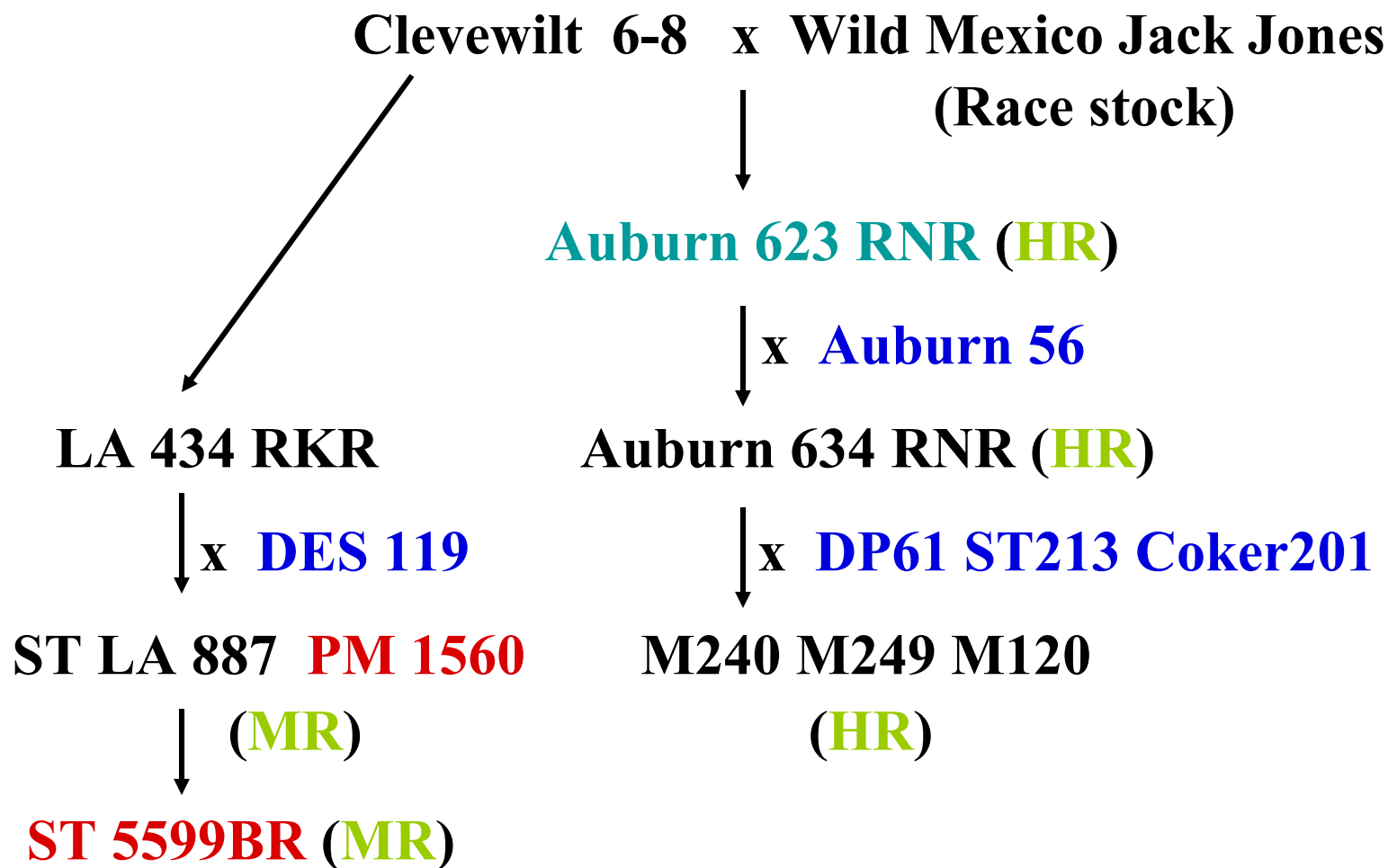


Root-Knot Nematode Resistance in Cotton: Research Progress at NMSU

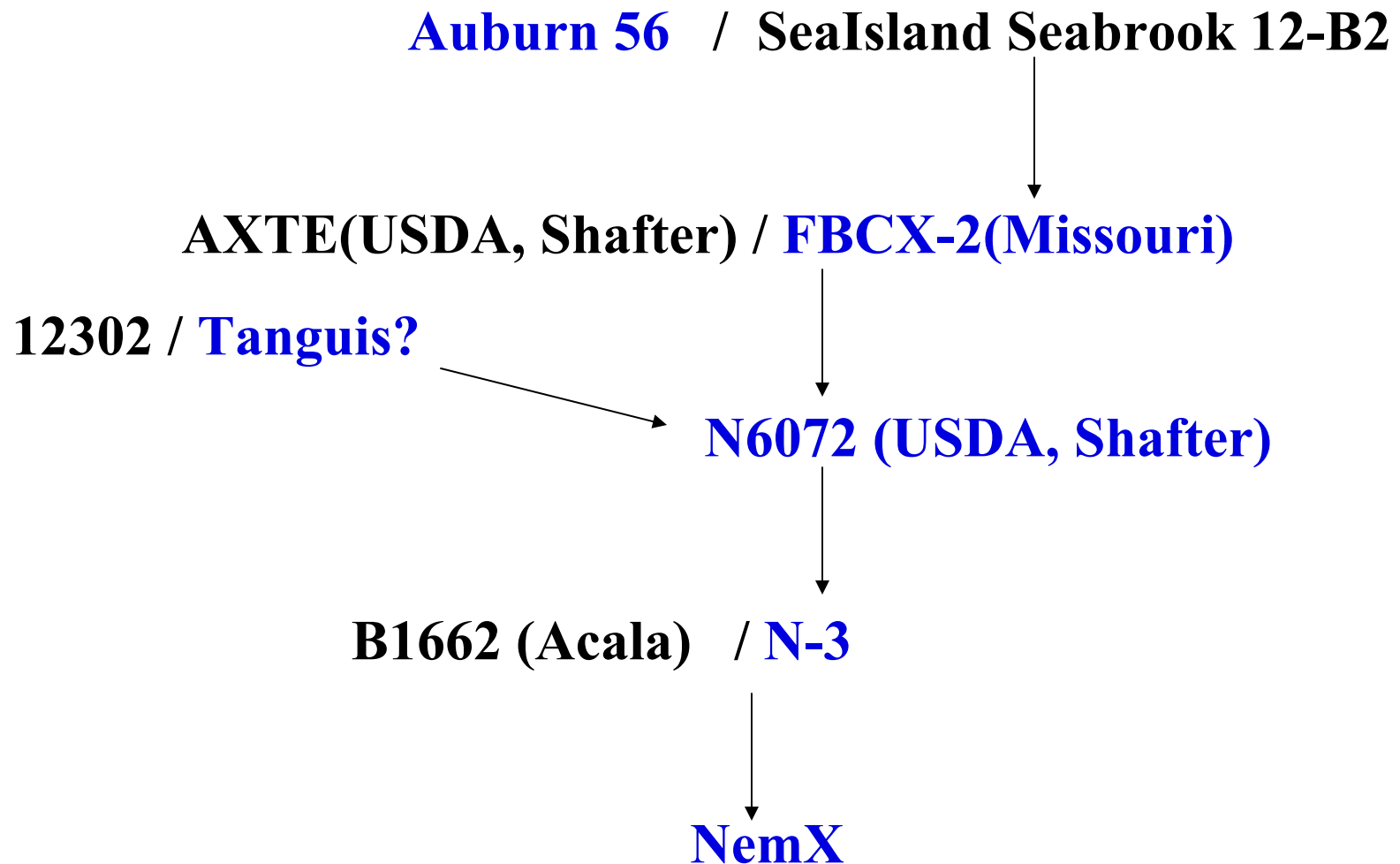
Jinfa Zhang

New Mexico State University

Major Cotton Germplasm Sources for RKN Resistance: Auburn 623

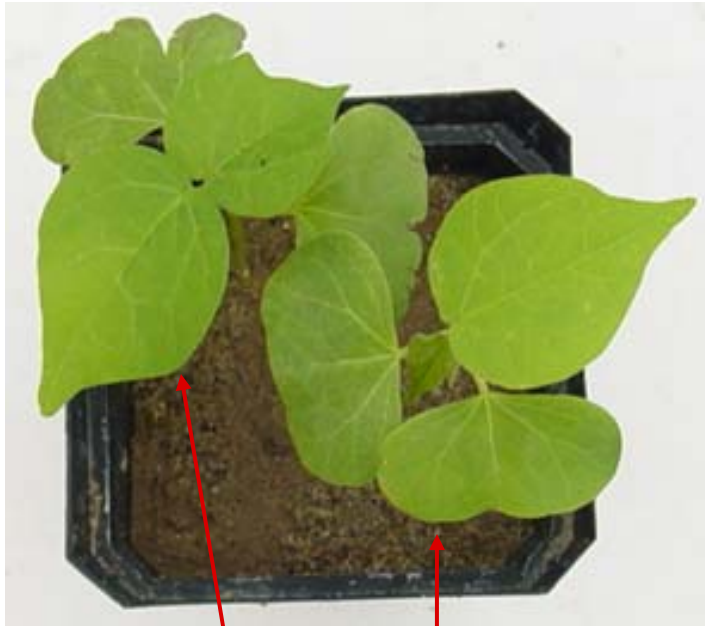


Major Cotton Germplasm Sources for RKN Resistance: Acala NemX



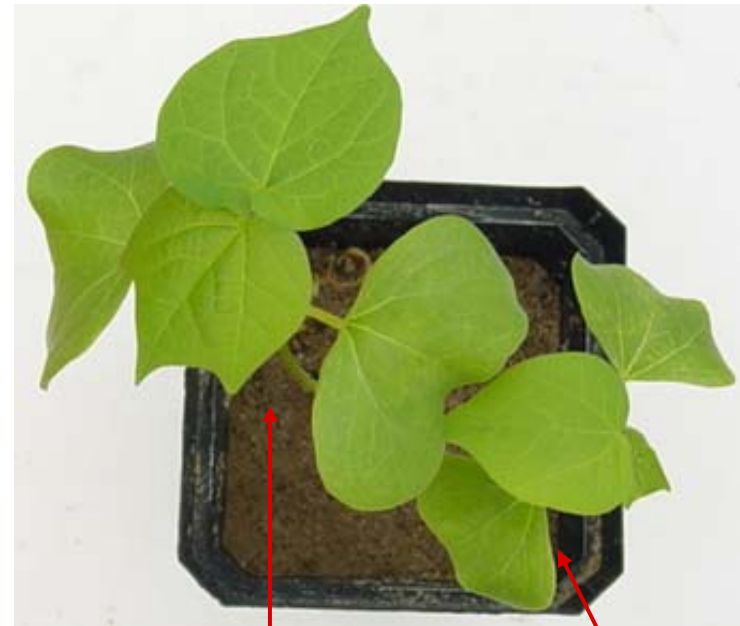
Galling Index

- | | |
|----------|--|
| 0 | No galls |
| 1 | Light galling (<25% roots with galls) |
| 2 | Moderate galling (20-50% root galling) |
| 3 | Heavy galling (50-75% root galling) |
| 4 | Very heavy galling (>75% root galling) |



ST 474

1517-99 (CK)



Auburn 634

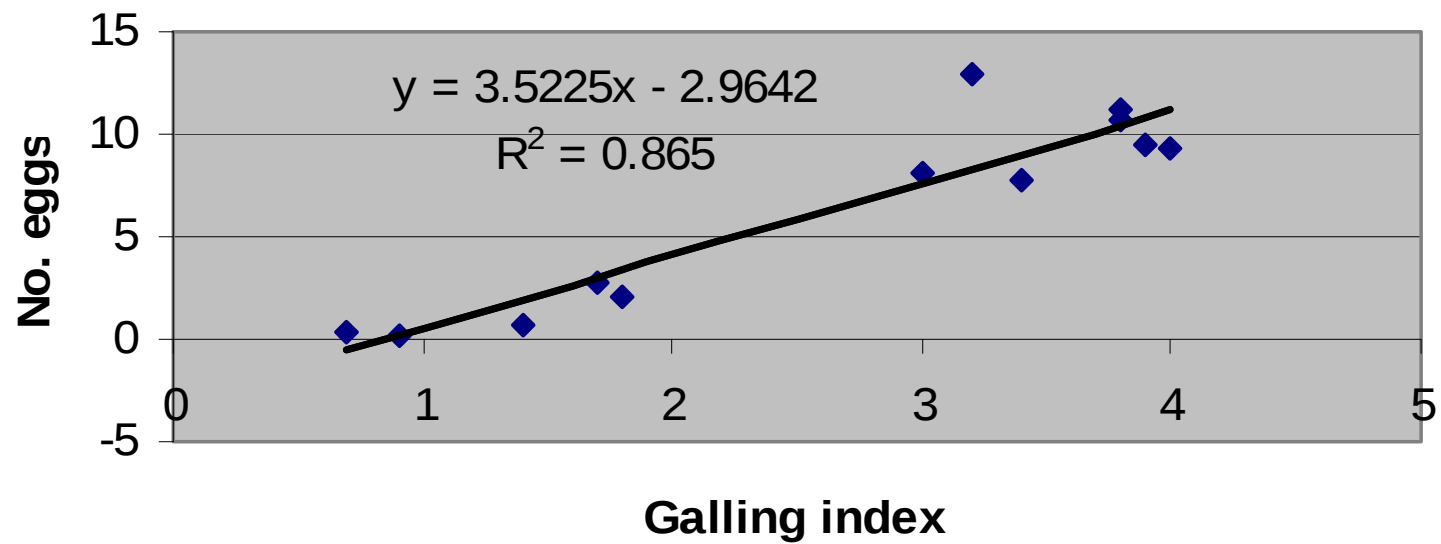
1517-99 (CK)



Genotypic Differences in RKN Resistance

Cultivar	Galling	Galling	Eggs x10 ⁵ /g root
33B	S	3.8	11.18
ST 474	S	3.9	9.49
SG 747	S	4.0	9.27
PM 1560BG	S	3.8	10.71
SG 125	S	3.2	12.97
DP 428BG	S	3.0	8.08
Maxxa	S	3.4	7.81
A 634	R	0.7	0.39
M 240	R	1.4	0.76
M 315	R	0.9	0.24
33B x A 634 F1	MR	1.8	2.14
NemX	MR	1.7	2.70
1517-99 (CK)	S	3.8	

Relationship between galling index and no. eggs

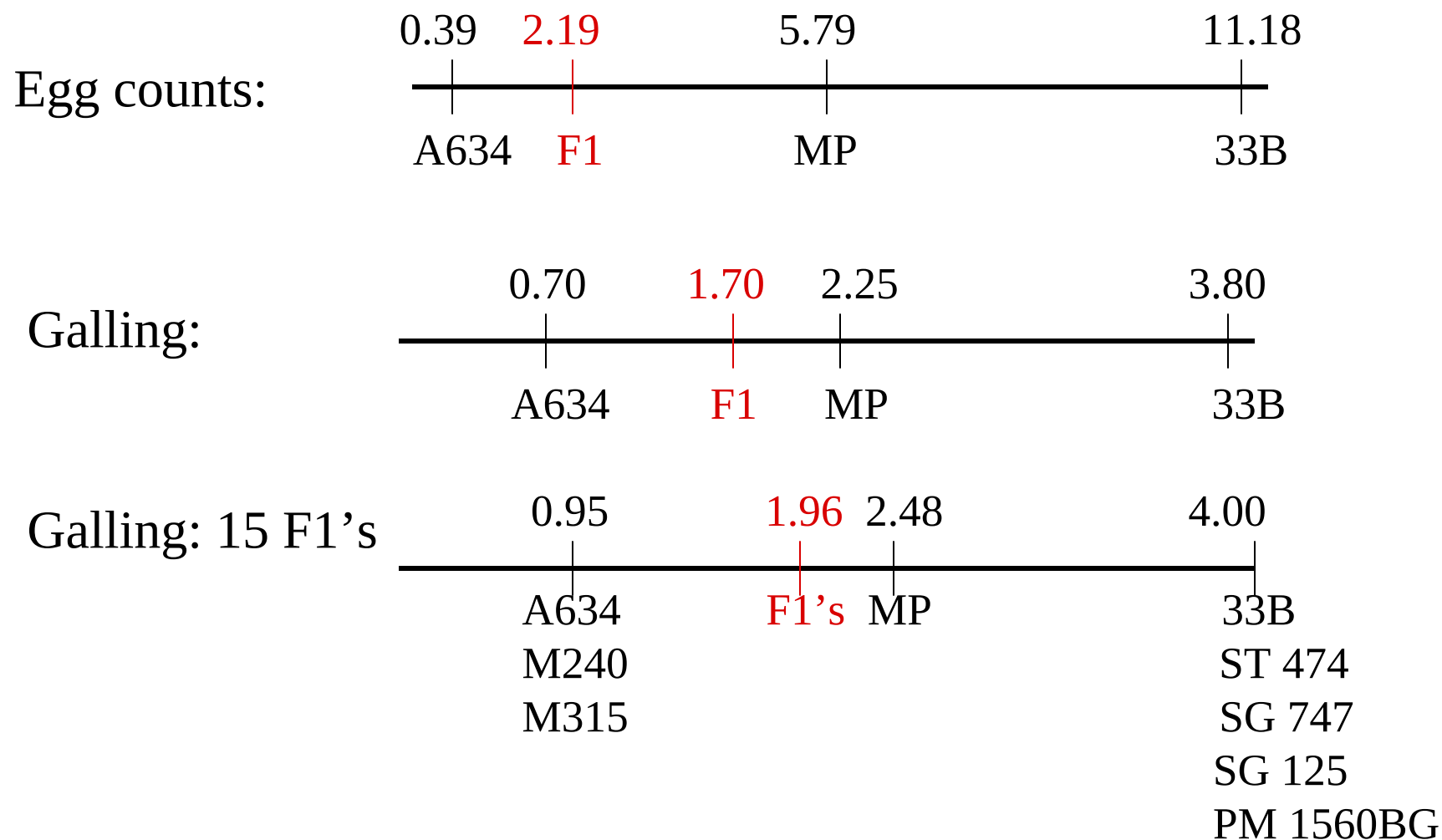


Correlation between Plant Growth and RKN Resistance

	Plant height	No. Leaves	Plant weight	Galling index	No. eggs/g
Plant height	1				
No. leaves	0.754*	1			
Plant weight	0.704*	0.549	1		
Galling index	-0.712*	-0.797*	-0.664*	1	
No. eggs/g	-0.755*	-0.817**	-0.683*	0.925**	1

N = 12

The Resistance in Auburn Source: Incomplete Dominance



Estimates of Genetic Parameters

Genetic parameter	Estimator
Broad-sense heritability: H_b	0.82
Narrow-sense heritability: H_n	0.65
Number of effective factors: K_1	0.30
Number of effective factors: K_2	1.17

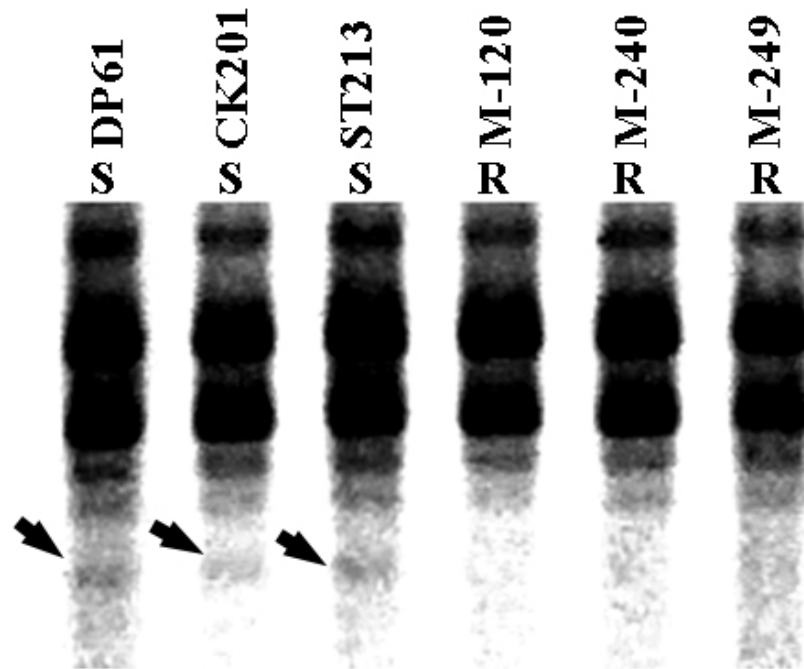
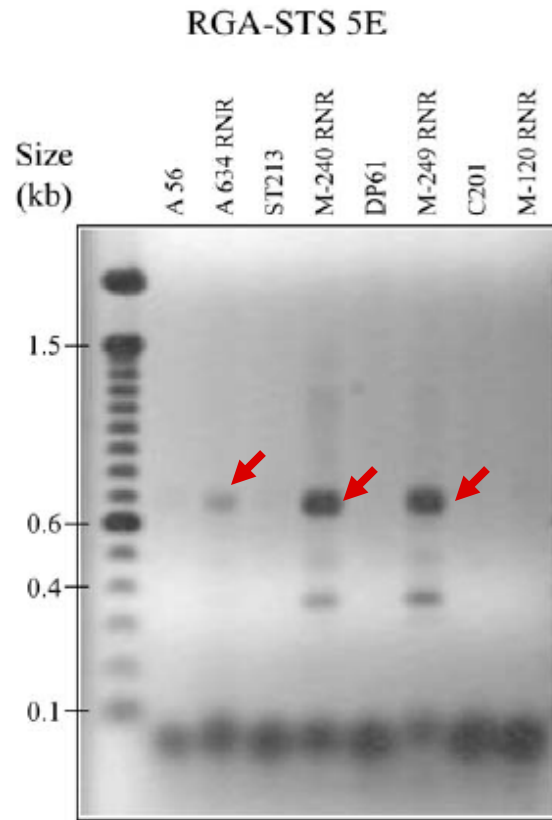
Segregation Data

Population	No. R	No. S.	Exp. ratio	χ^2
1517-99 x ST 5599 F3	60	22	3:1	0.15
Nem-X x Auburn 634 F3	168	0	15:1	11.2**

Controversial but Clearer Picture

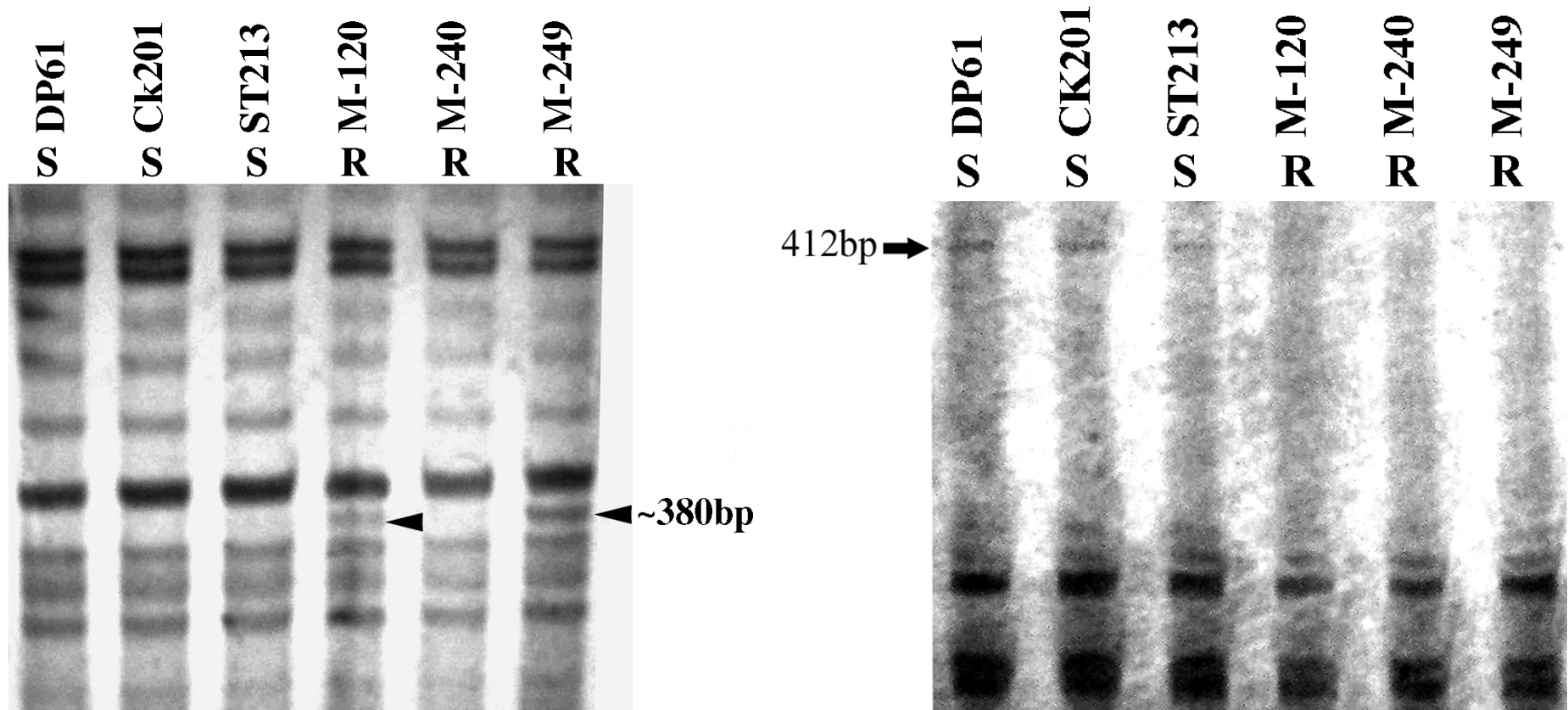
- **Auburn source: WM Jack Jones x Cleviewilt → Auburn 623**
 - Shepherd (1974): polygenic and partially dominant
 - Jenkins's lab (McPherson, 1993): 1 dominant & 1 additive
 - Starr's lab (Zhou, 1999): 2 dominant genes
 - McPherson et al. (2004): two genes, *Mi-1* & *Mi-2*
 - **G.h. race stocks and conversions:**
 - McPherson (1993) & M 19-- 1 additive
 - McPherson et al. (2004): M 78-- 1 dominant, *Mi-1*
M 75-- 1 dominant & 1 additive
 - **Cleviewilt: *Mi-2*?**
 - Benzawada et al. (2003): 1 recessive gene
 - Zhou (1999): LA 887 (derived from Cleviewilt) - 1 recessive gene = Nem-X, *rkn1*
 - Present study: ST 5599BG (same as LA 887) - 1 incomplete dominant gene
- **Nem-X:**
 - Starr's lab (Zhou, 1999): 1 recessive gene
 - Roberts' lab: single gene, *rkn1* mapped to LG A03
 - Present study: possible one of the two genes in the Auburn source, *Mi-2*

Auburn 623 Source

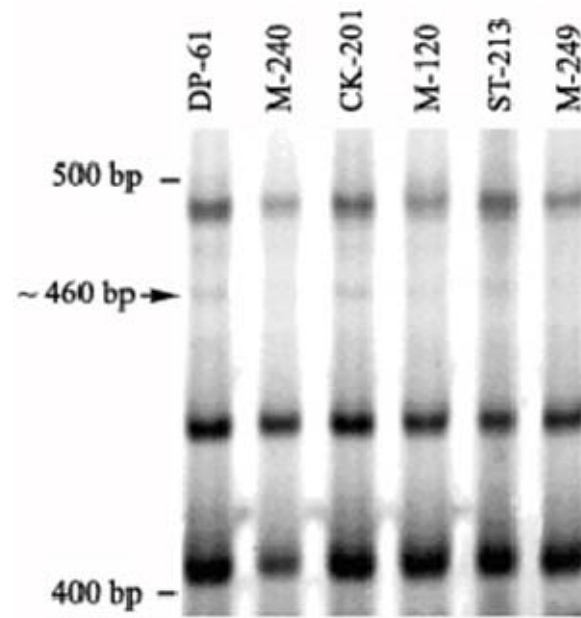
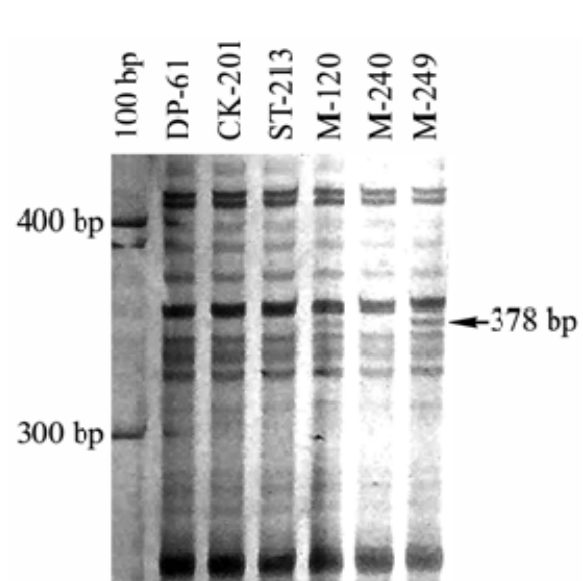


Genomic DNA from three pairs of NILs amplified with resistance gene analog (RGA) primers RGA-STS 5E and RLK.

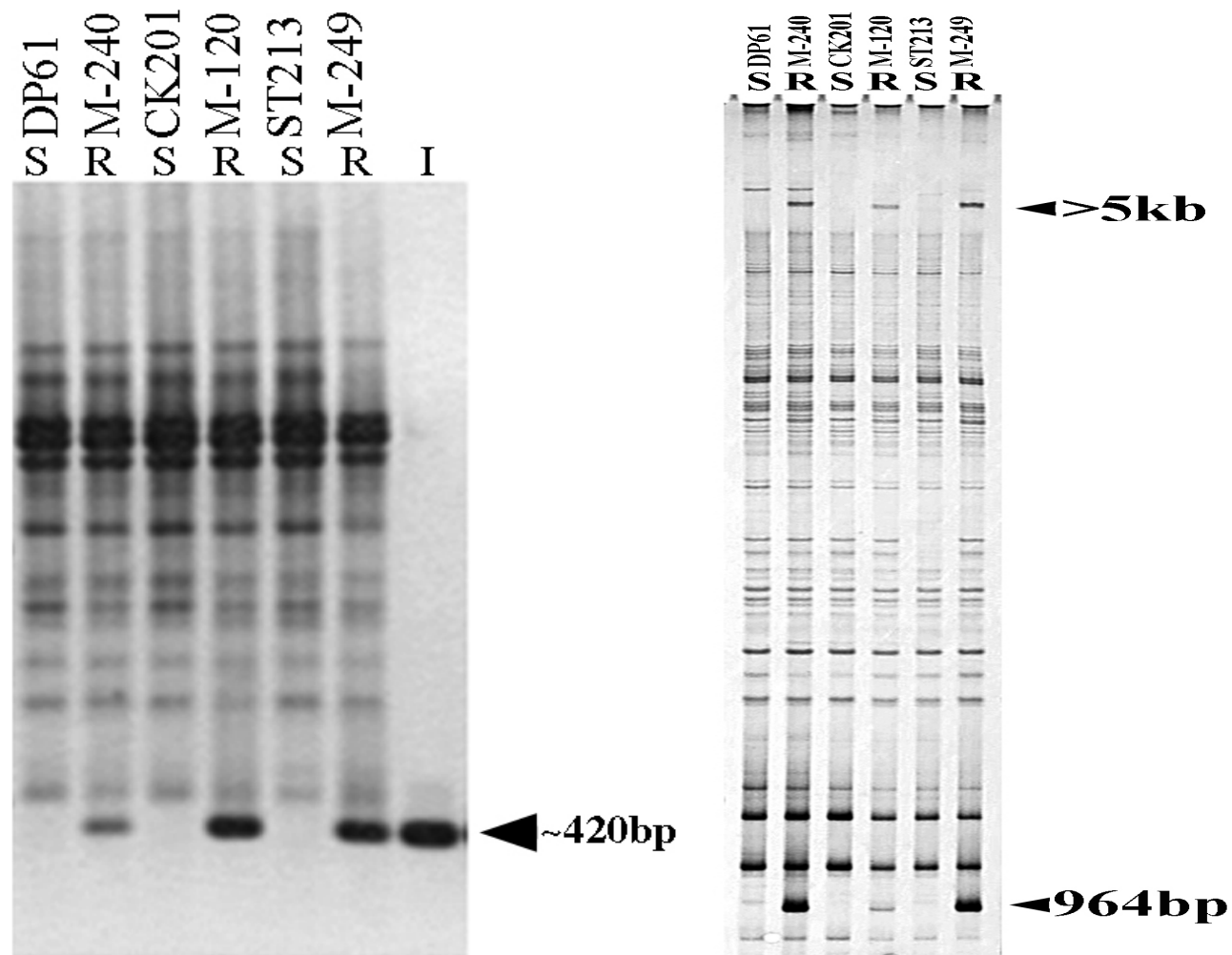
Auburn 623 Source



Two AFLP polymorphic markers amplified by primer pair B1 and C3 from genomic DNA isolated from near-isogenic lines resistant or susceptible to RKN.

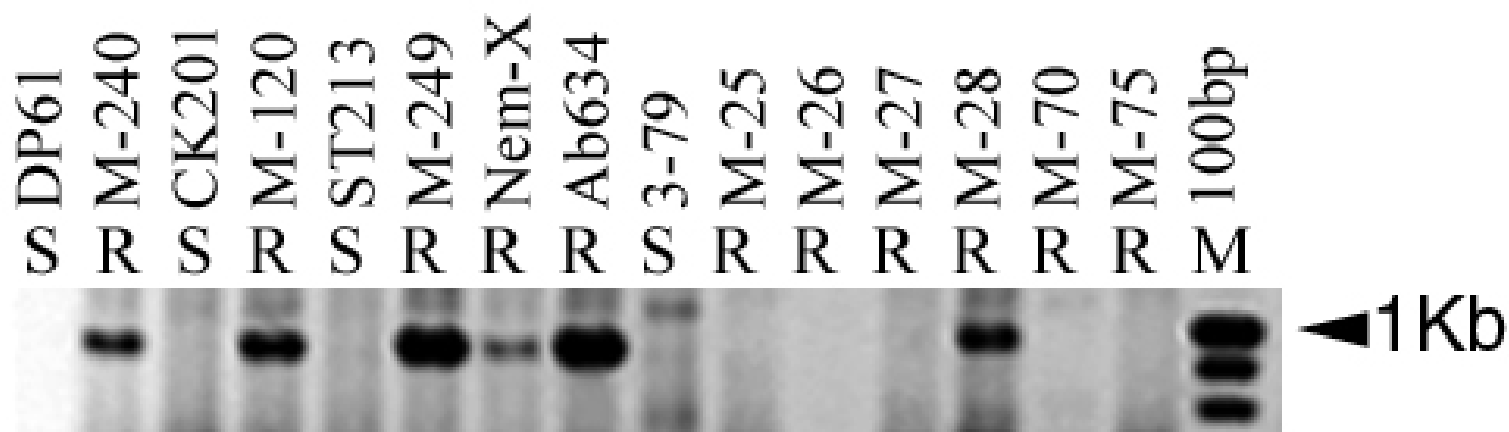


Auburn 623 Source

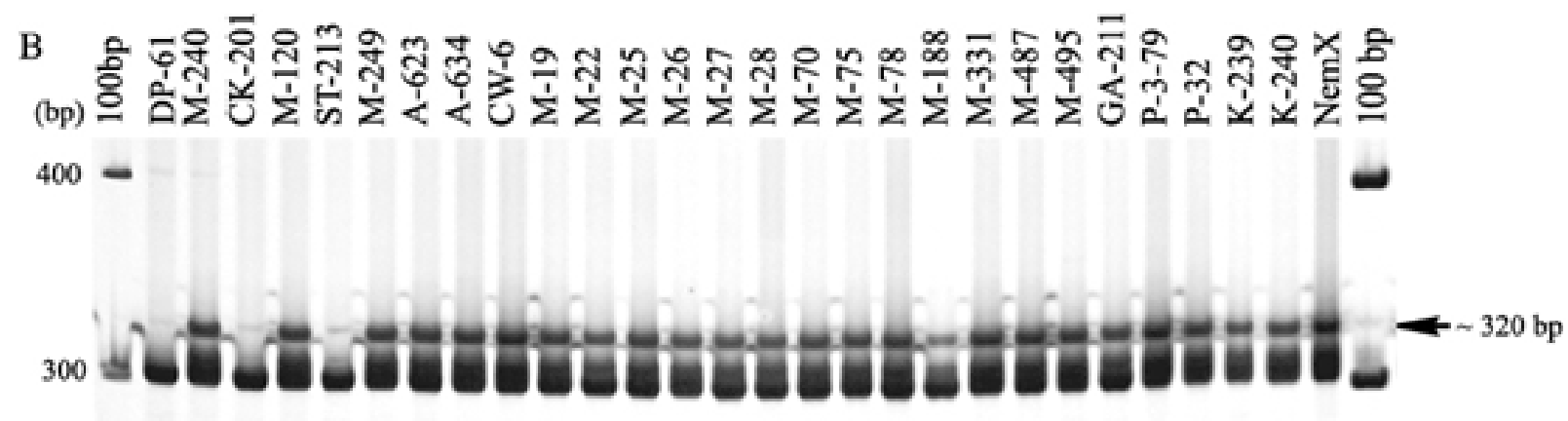
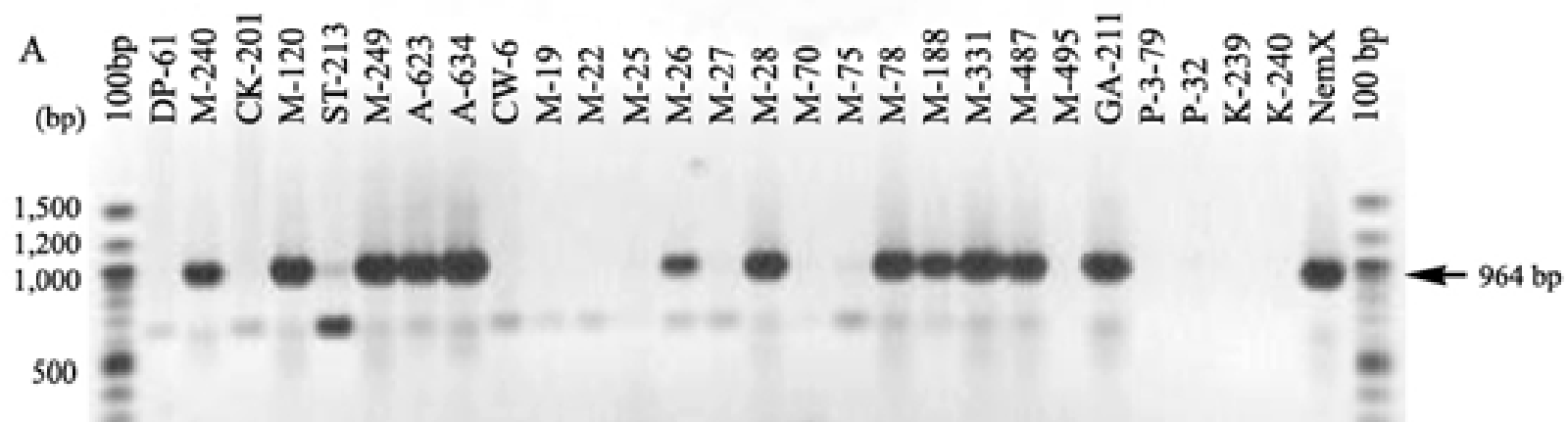


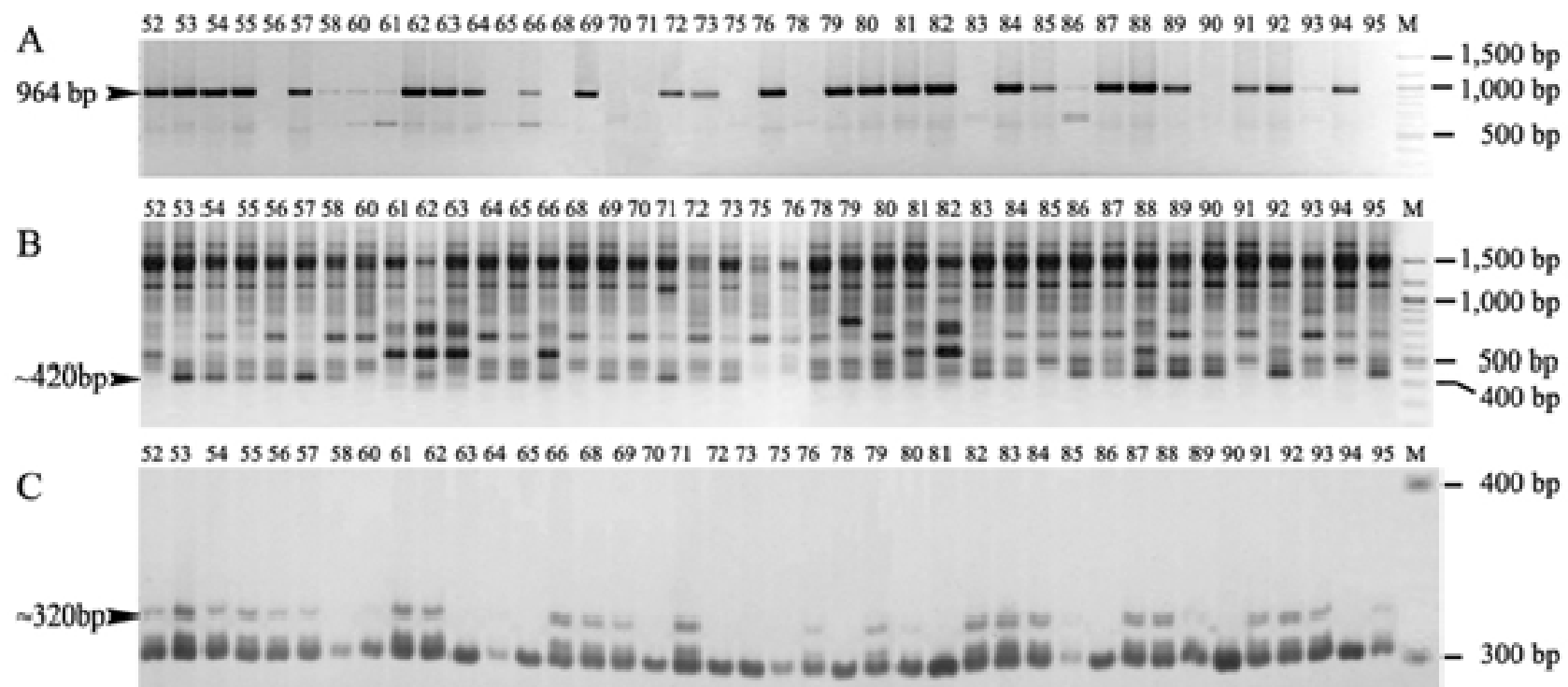
Three RAPD markers (NMRKN6931-420, NMRKN7811-1000, and NMRKN7811-5000) associated with cotton resistance to RKN.

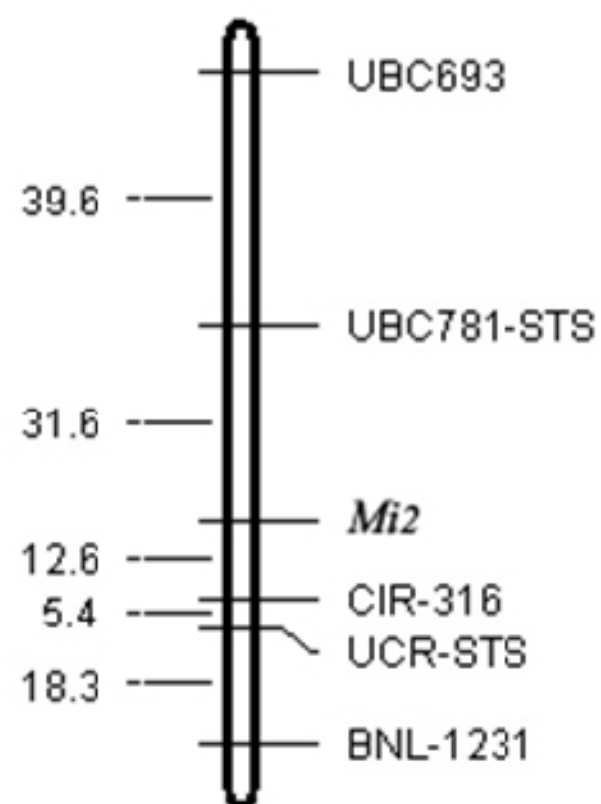
Auburn 623 Source



A sequence tagged site (STS) marker (NMRKNR7811-1000 STS) amplified by STS primers designed from the NMRKN7811-1000 fragment. It is absent in all the susceptible lines and present in all the Auburn lines, Nem-X, and some converted race stocks.







Germplasm Screening

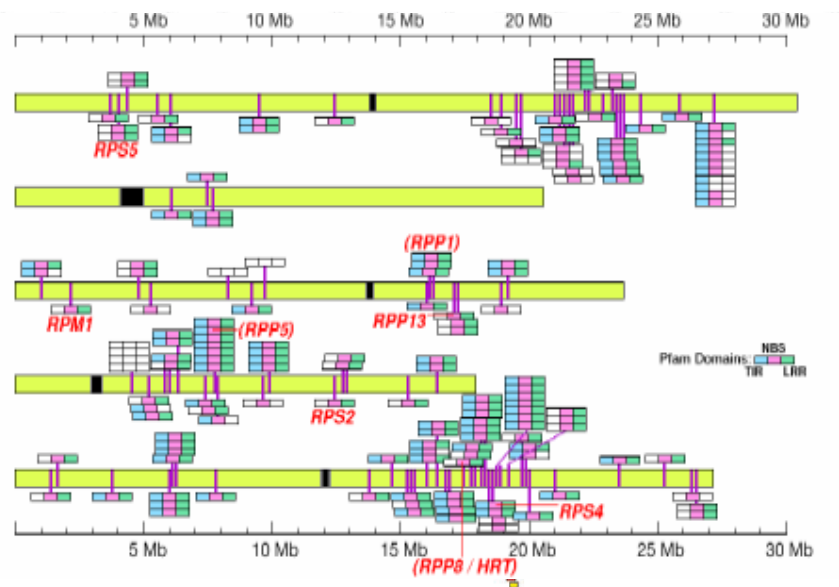
Genotype	PI No.	Pedigree	Reaction to RKN	UBC 693-420	UBC 781-1000	UBC 781-964 STS	UCR-STS	CIR 316	Group
DP 61			S	-	-	-	-	-	3
M-240	592511	AB 634 RNR / DP61	R	+	+	+	+	+	1
CK 201			S	-	-	-	-	-	3
M-120	592509	AB 634 RNR / CK201	R	+	+	+	+	+	1
ST 213			S	-	-	-	-	-	3
M-249	592512	AB 634 RNR / ST213	R	+	+	+	+	+	1
AB 623 RNR	SA 1492		R	+	+	+	+	+	1
AB 634 RNR	GP 166	AB 623 RNR / AB 56	R	+	+	+	+	+	1
Clelewilt 6	SA 245		?	-	-	-	+	+	3
M-19 RNR	517931	<i>Richmondii</i> / DP 16	R	+	-	-	+	+	2
M-22 RNR	517932	<i>Latifolium</i> / DP 16	R	+	-	-	+	-	2
M-25 RNR	517933	<i>Punctatum</i> / DP 16	R	+	-	-	+	-	2
M-26 RNR	517934	<i>Punctatum</i> / DP 16	R	+	-	-	+	-	2
M-27 RNR	517927	<i>Punctatum</i> / DP 16	R	+	-	-	+	-	2
M-28 RNR	517928	<i>Punctatum</i> / DP 16	R	+	+	+	+	-	1
M-70 RNR	517930	<i>Latifolium</i> / DP 16	R	+	-	-	+	+	2
M-75 RNR	517929	<i>Latifolium</i> / DP 16	R	+	-	-	+	+	2
M-78 RNR	517930	<i>Latifolium</i> / DP 16	R	+	+	+	+	+	1
M-188 RNR	517936	<i>Latifolium</i> / DP 16	R	+	+	+	+	-	1
M-331 RNR	592515	AB 634 RNR / AB56	R	+	+	+	+	+	1
M-487 RNR	517937	<i>Punctatum</i> / DP 16	R	+	+	+	+	+	1
M-495 RNR	517938	<i>Punctatum</i> / DP 16	R	+	-	-	+	-	2
M-725 RNR	592516	AB 634 RNR / CK310	R	+	-	-	+	+	2
GA 96-211	633019	GA77-27/'PD-3'//GA88-92/3/M-240-RNR/4/M-120-RNR/5/'LA887'	R	+	+	+	+	+	1
Wild Mexican	TX 2516		?	+	-	-	+	-	2
Jack Jones									
Pima 3-79			S	-	-	-	+	-	3
NM 24016			S	-	-	-			3
Pima 32			S	-	-	-	+	-	3
SxP			S	-	-	-	+	-	3
Amsak			S	-	-	-	+	-	3
Acala NemX			R	+	+	+	+	+	1

What Do We Know About Plant Genomes?

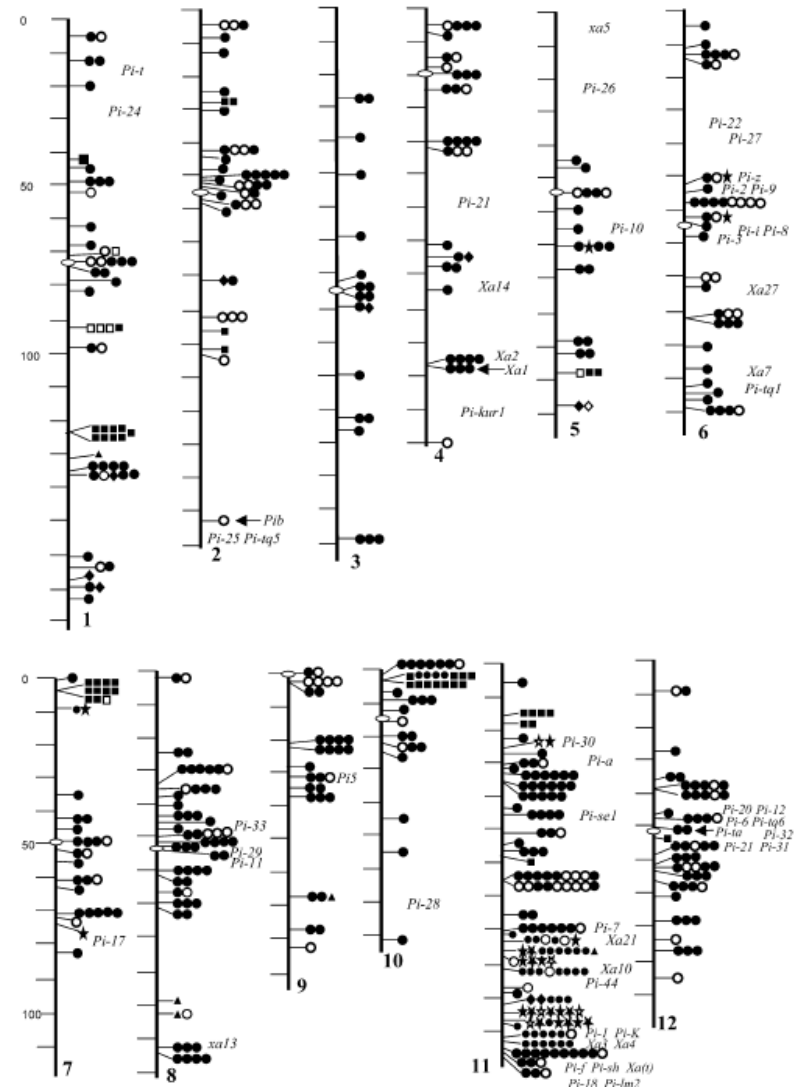
- **Coding sequences: gene families (~25%)**
 - **Arabidopsis**
 - ~26,000 genes
 - 867 gene families
 - 6457 genes (~ ¼ of total number of genes)



NBS-LRR Genes in Arabidopsis (Meyers et al., 2003)



NBS Genes in Rice (Monosi et al., 2004)



RGA-AFLP Analysis



Genomic DNA is **digested** with a frequent and a rare cutting enzyme (*Mse*I and *Eco*R1)



Three types of restriction fragments are generated: ones with *Eco*R1 cut at both ends, ones with *Eco*R1 cut at one end and *Mse*I cut at other end and ones with *Mse*I cuts at both ends.

*Eco*R1 adapter



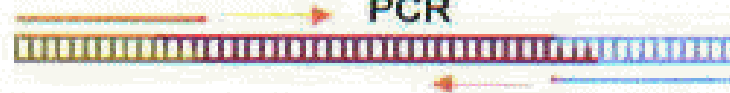
*Mse*I adapter



Specific adapters to restriction sites are then **ligated** to generated fragments.

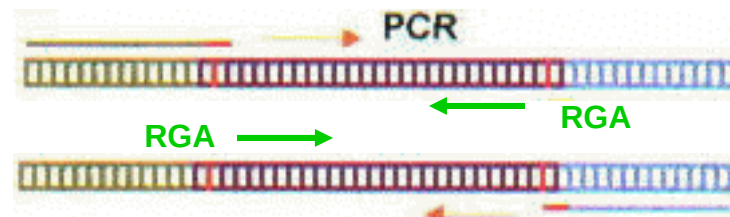
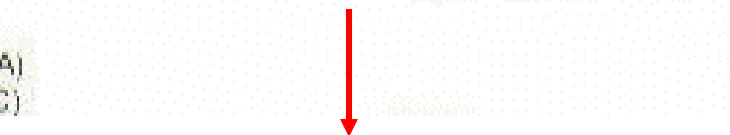


PCR

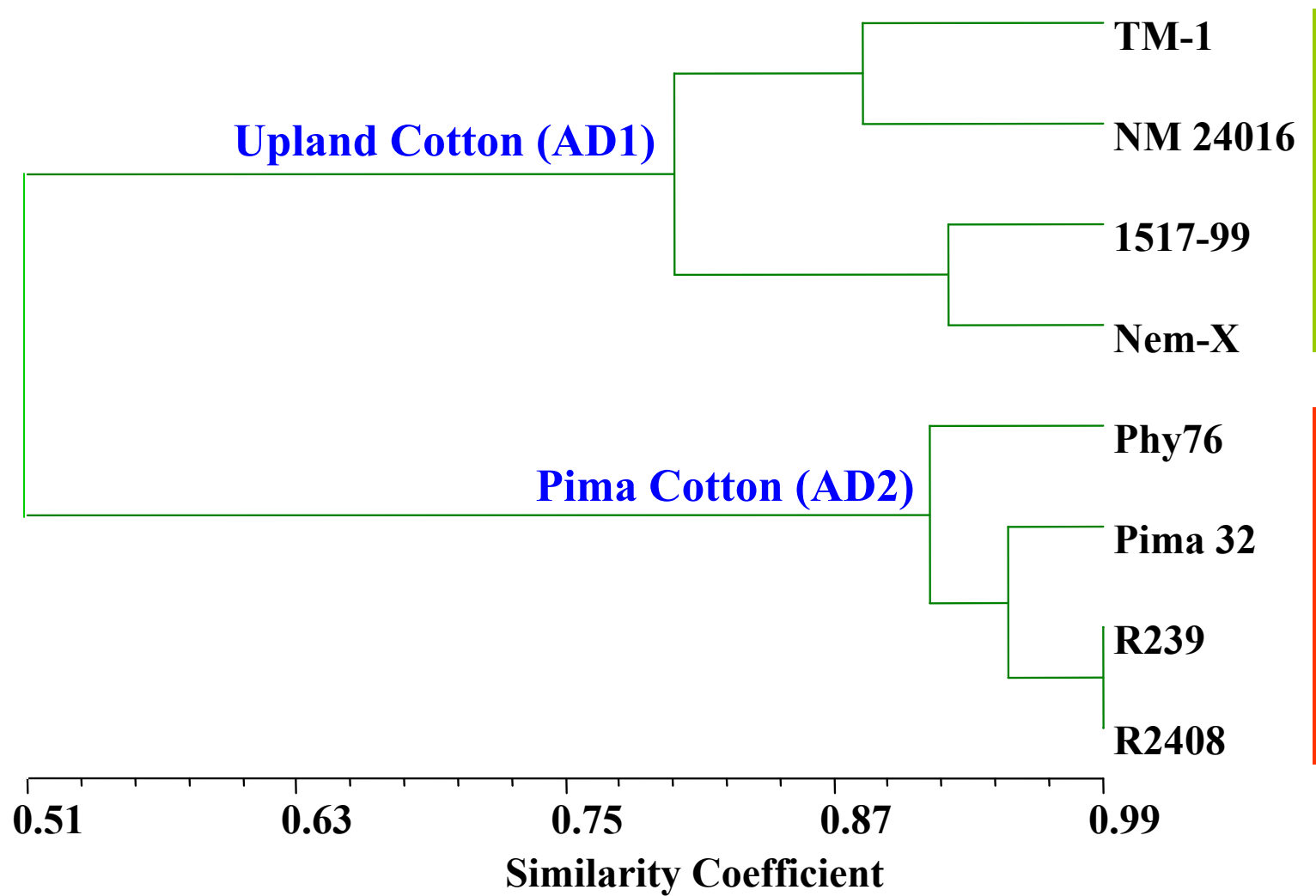


Preselective amplification step achieves a 16-fold reduction of the complexity of the restriction – ligation products

+1 Selective primers
(*Eco*R1 adapter + selective base A)
(*Mse*I adapter + selective base C)

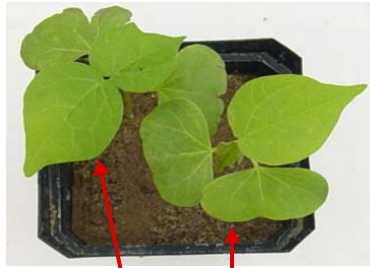


RGA-AFLP Analysis

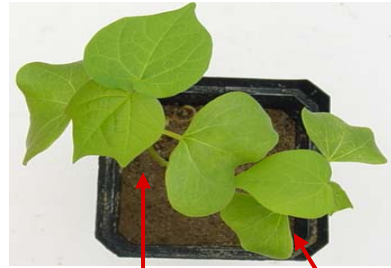


Cluster analysis based on RGA-AFLP

Root-knot Nematode Resistance



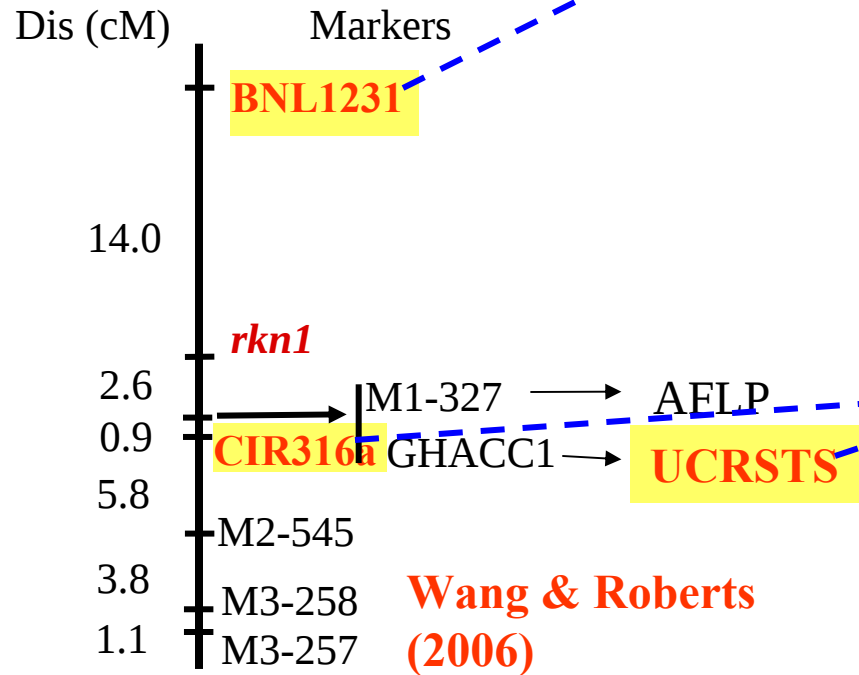
ST 474 1517-99 (CK)



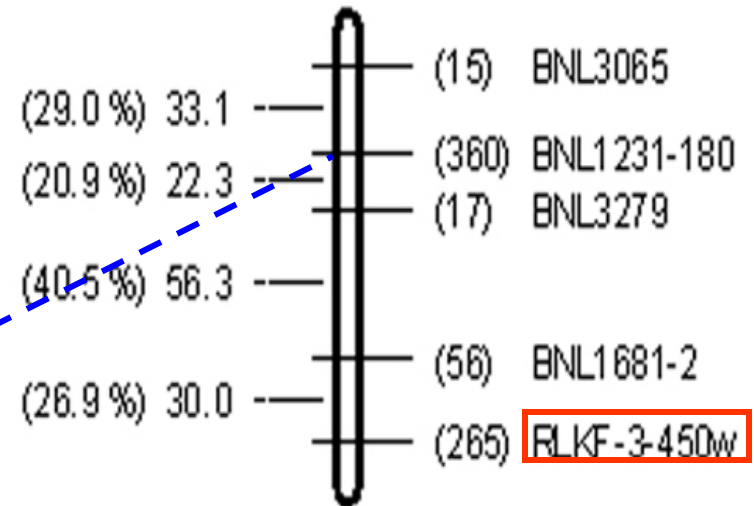
Auburn 634 1517-99 (CK)



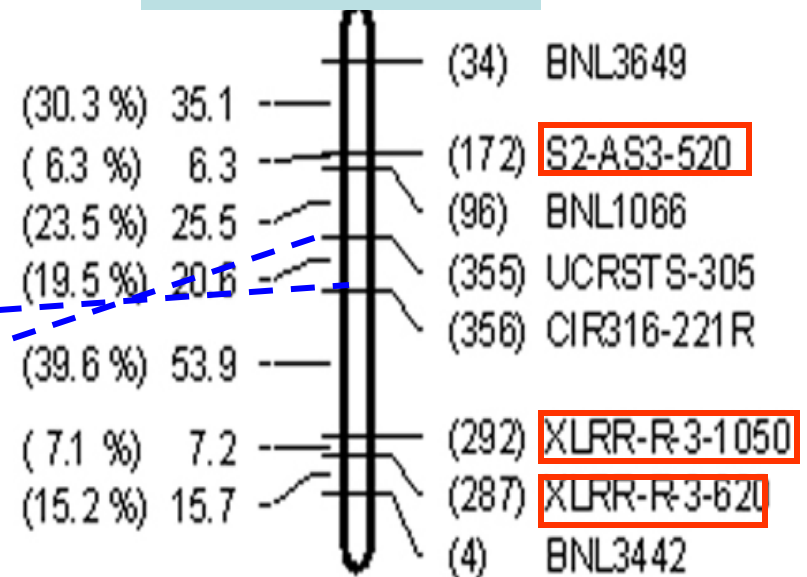
Chromosome 11

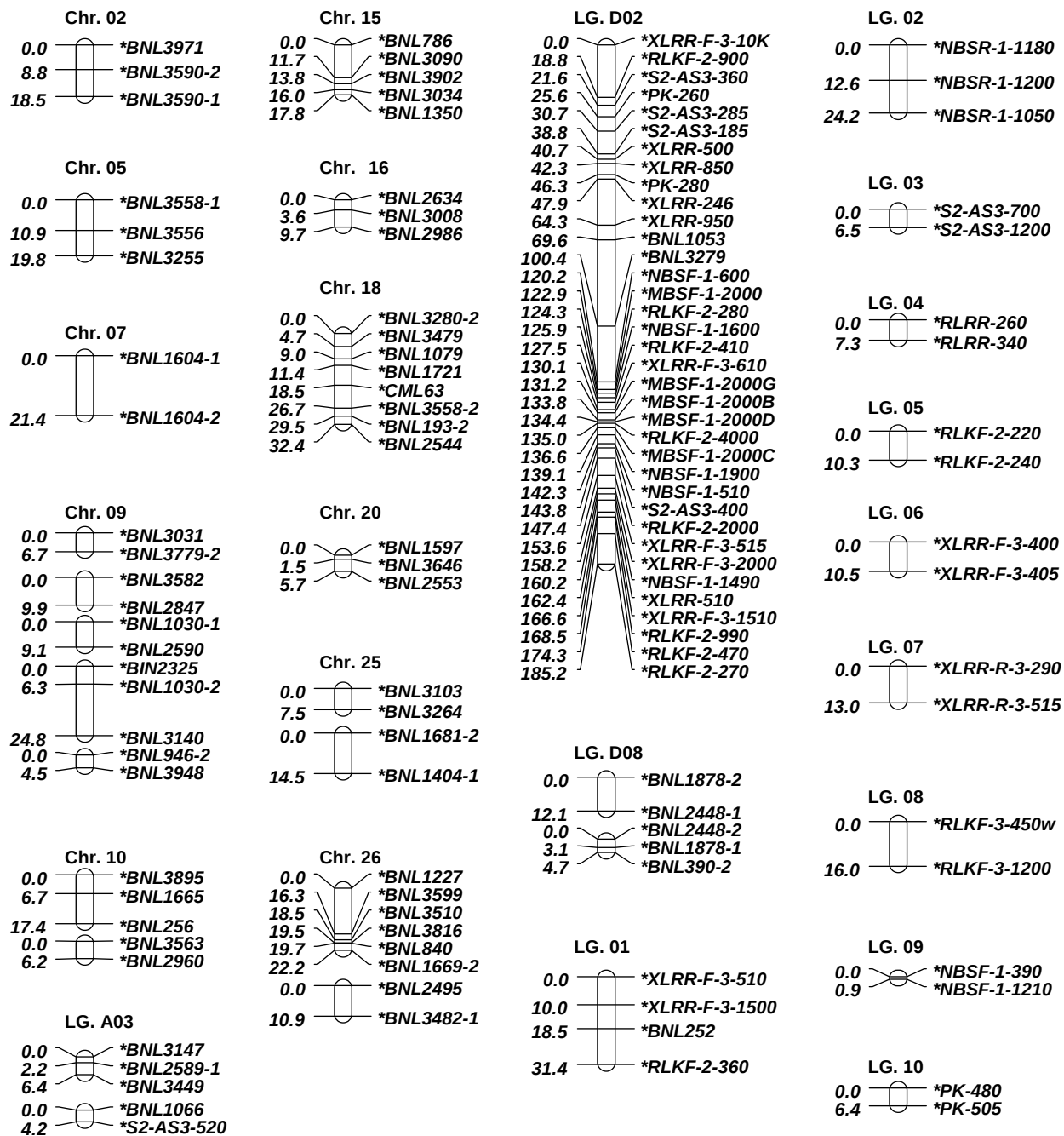


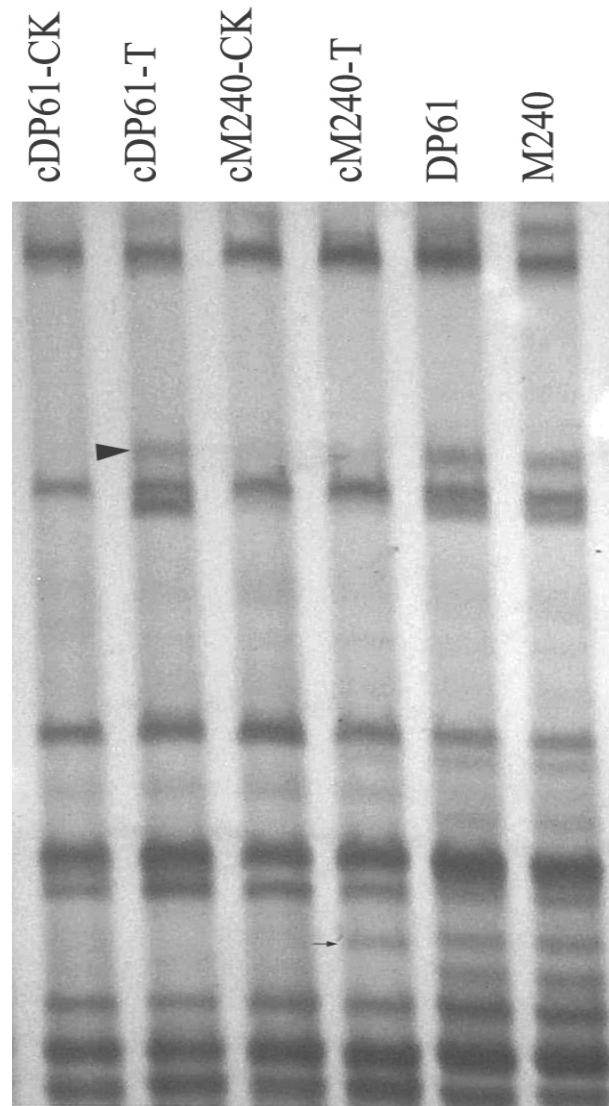
Chromosome 22



Chromosome 11







cDNA-AFLP analysis

Marker Screening

Line	RAPD 6911	RAPD 7811	STS 7811- 1000	UC- CAP	CIR 316a
Auburn source <i>Mi-1, Mi-2</i>	+	+	+	+	+
Most race stocks <i>Mi-1?</i>	+	-	-	-	-
W M Jack Jones <i>Mi-1?</i>	+	-	-	+	-
Clevewilt 6 <i>Mi-2?</i>	-	+	+	+	+
La lines <i>Mi-2?</i>	?	?	?	+	+
NemX <i>rkn1=Mi-2?</i>	+	+	+	+	+
Susceptible lines	-	-	-	-	-