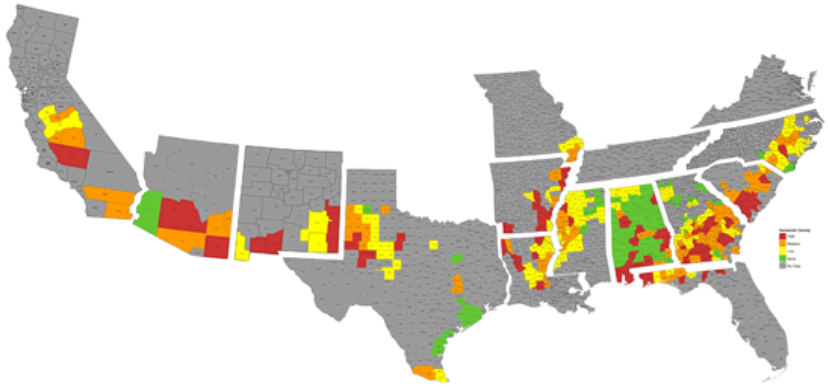
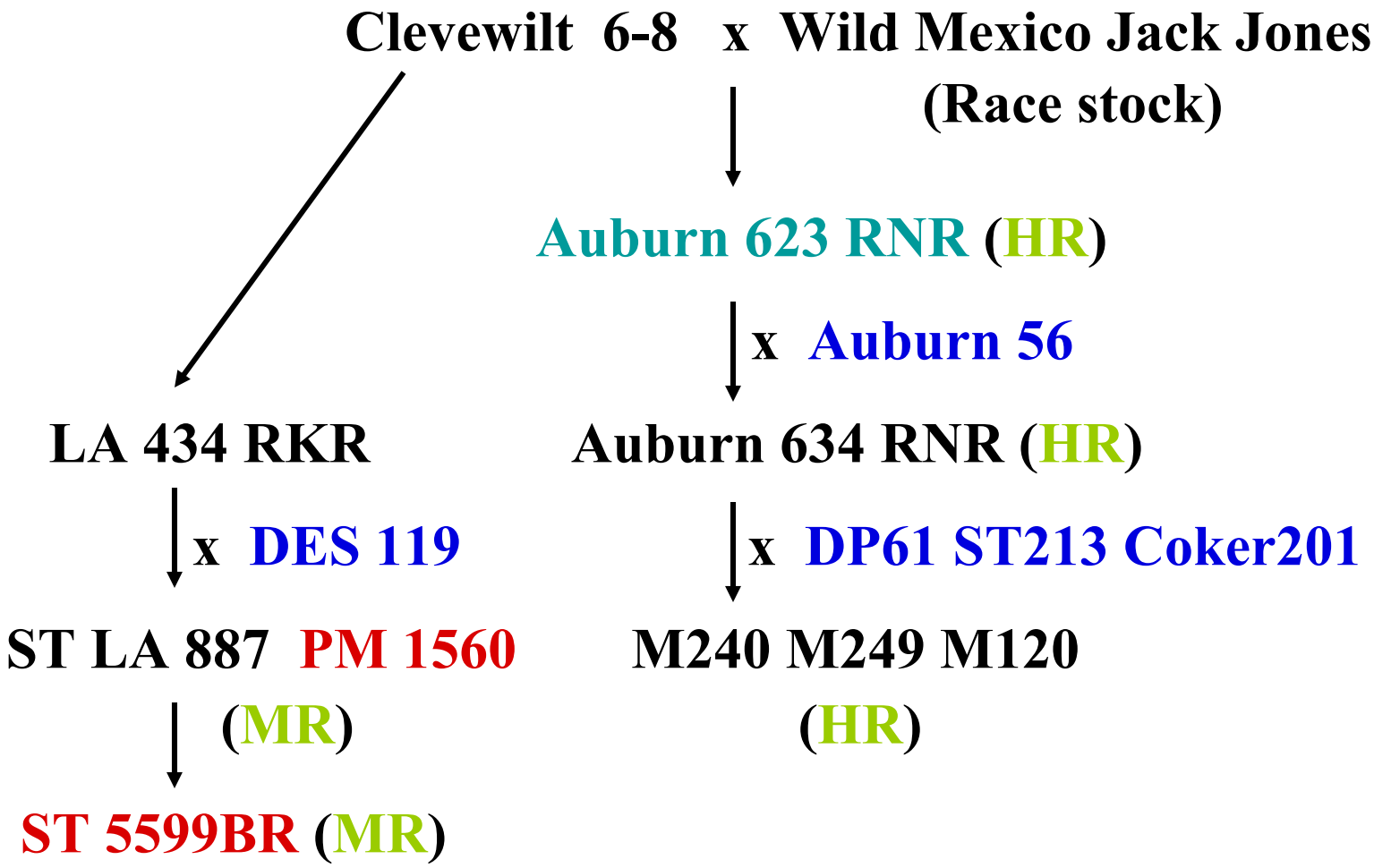


Root-Knot Nematode Resistance in Cotton: Research Progress at NMSU

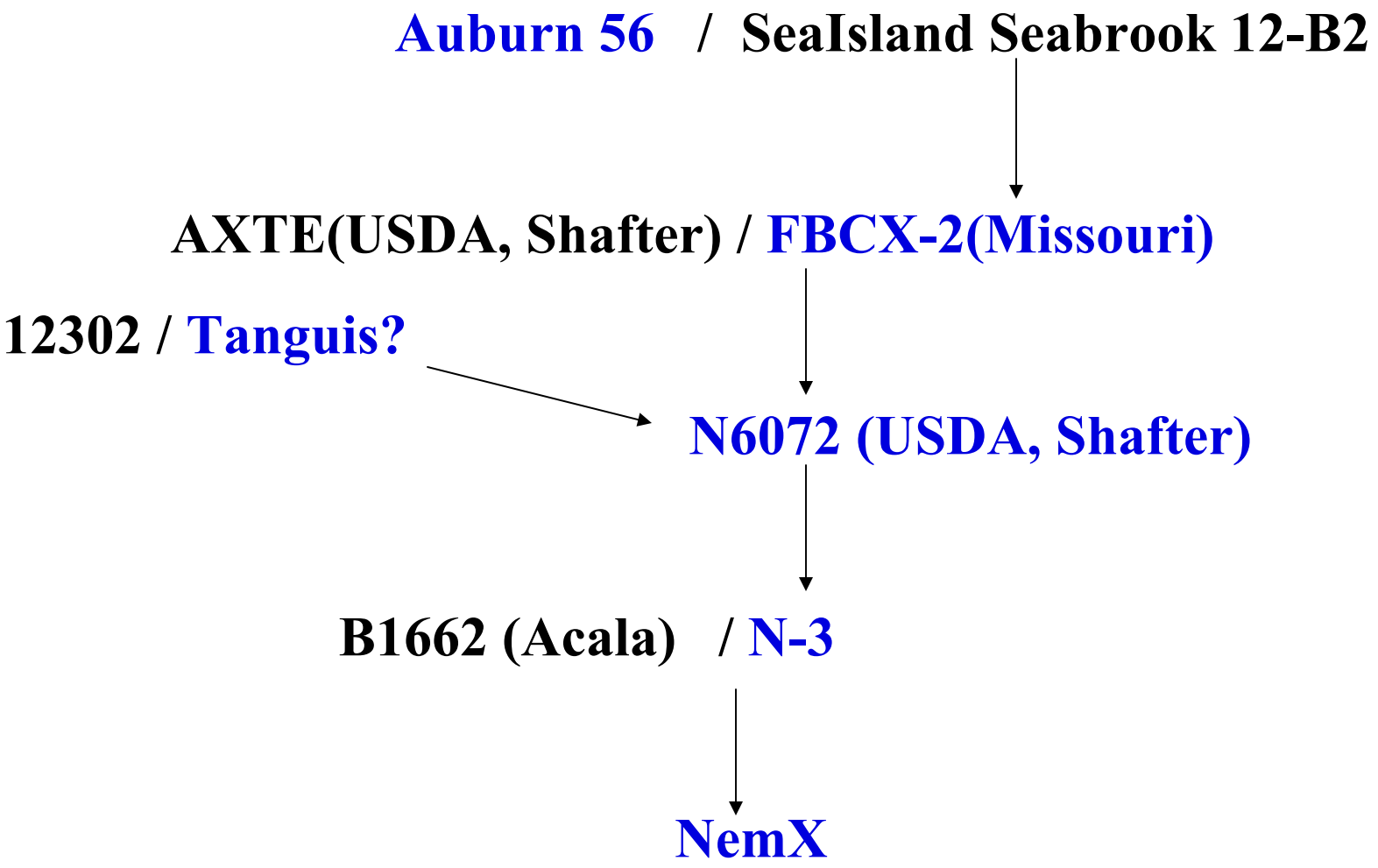
Jinfa Zhang

New Mexico State University

Major Cotton Germplasm Sources for RKN Resistance: Auburn 623

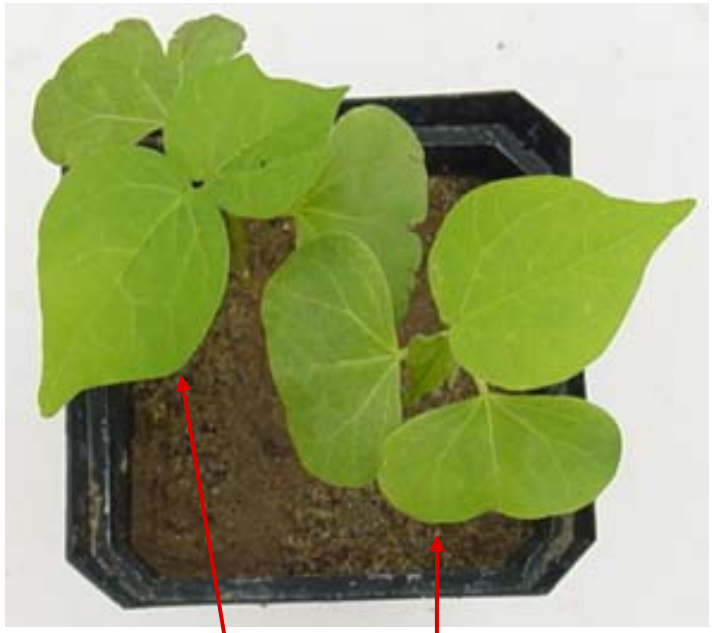


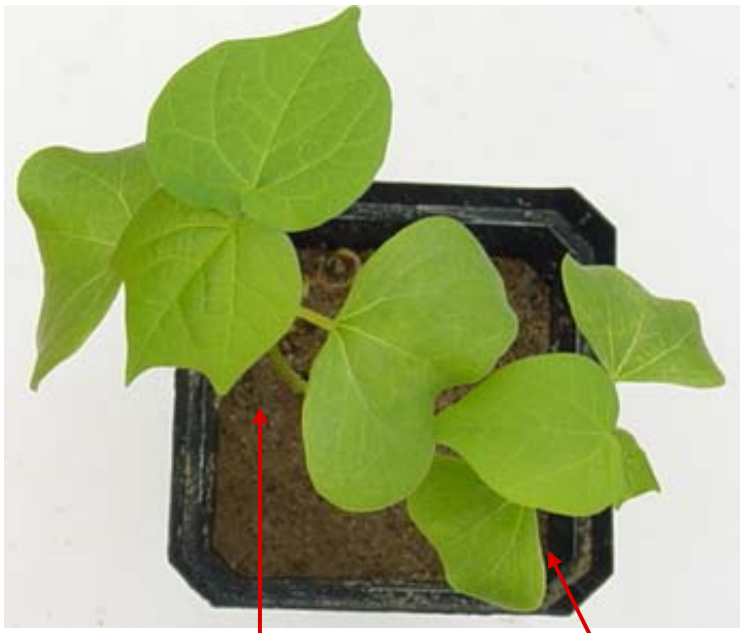
Major Cotton Germplasm Sources for RKN Resistance: Acala NemX



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)

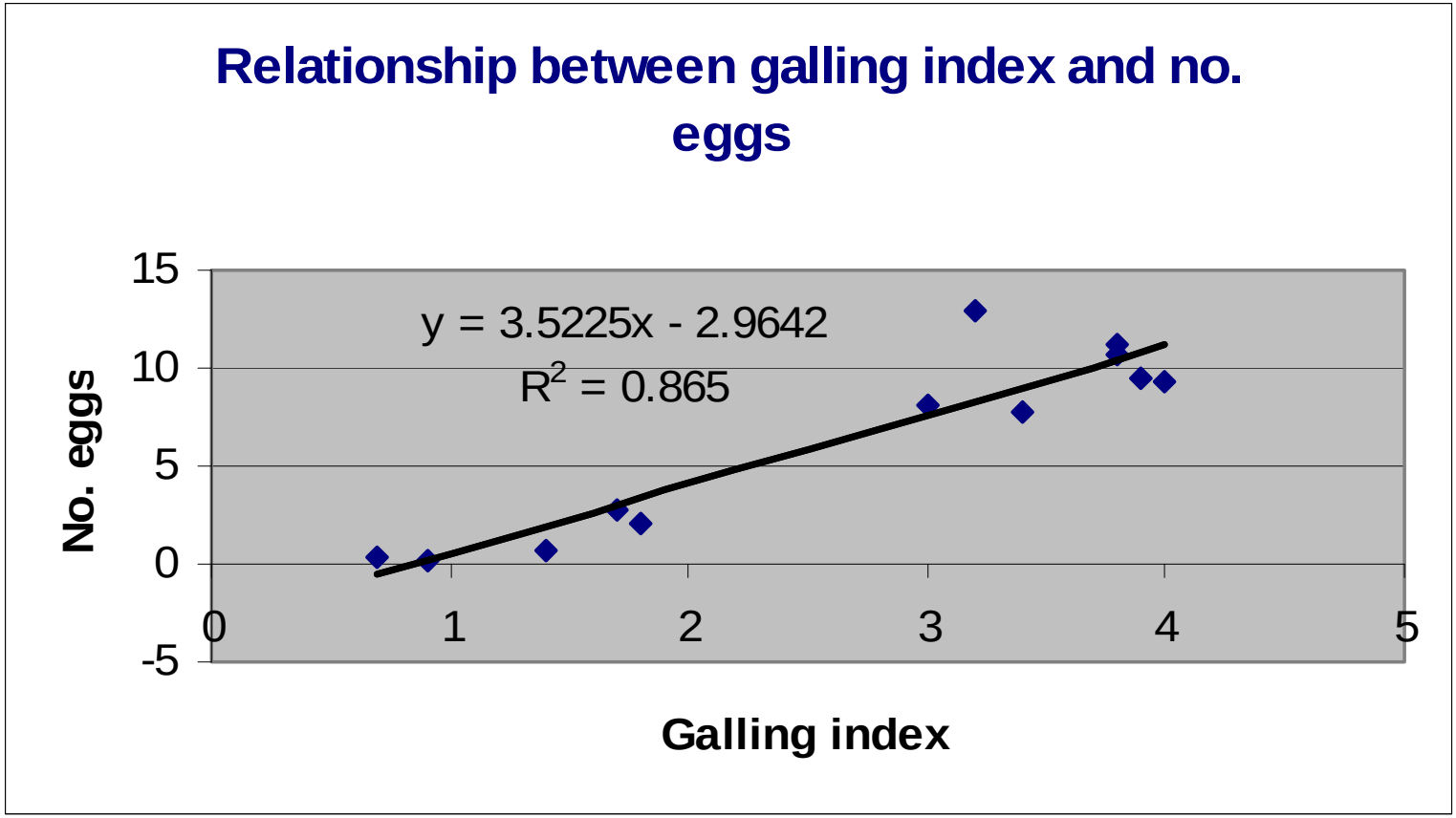




ST 474 1517-99 (CK) Auburn 634 1517-99 (CK)

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	

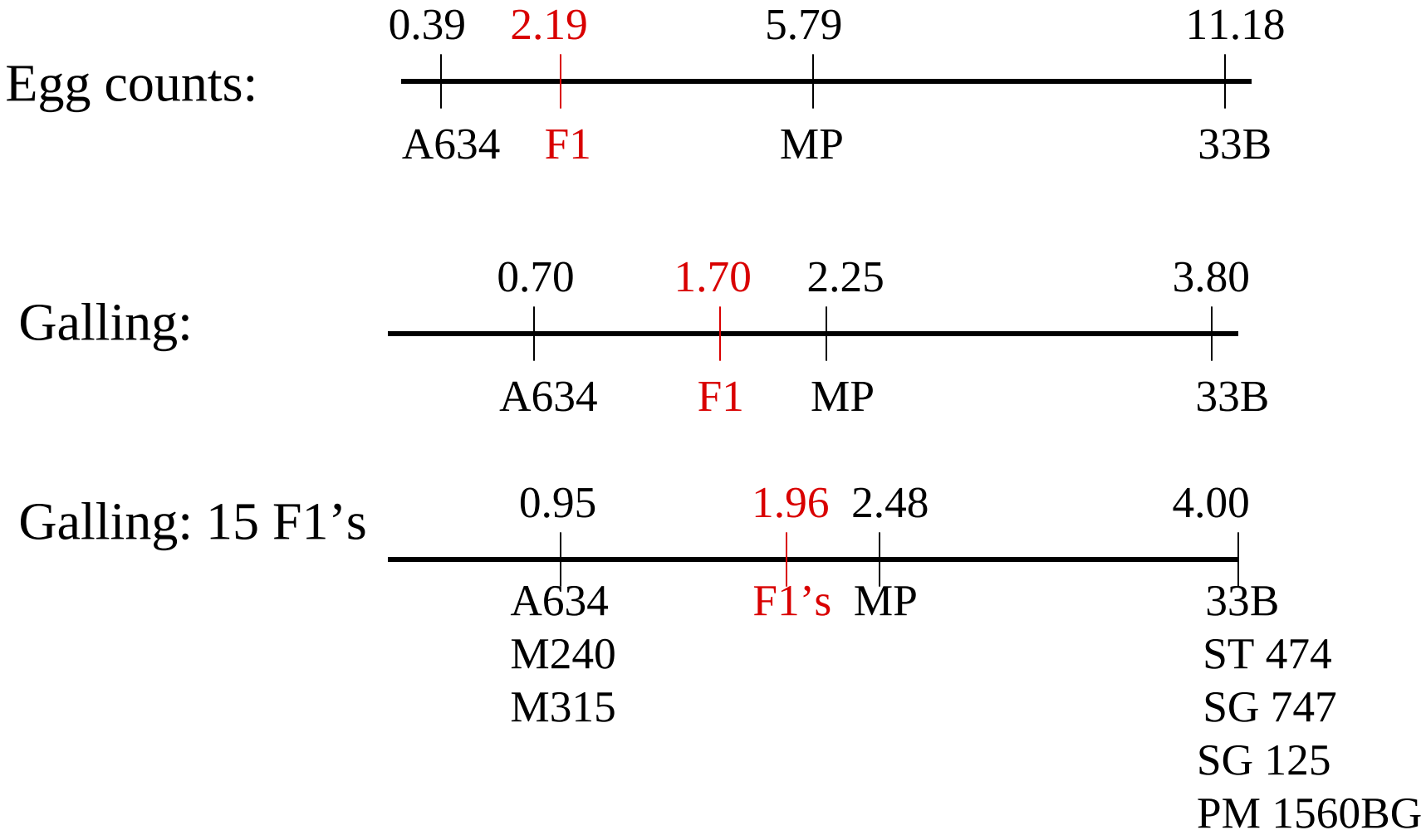

Relationship between galling index and no. eggs
y = 3.5225x - 2.9642
R2 = 0.865
No. eggs
15
10
5
0
-5
0
1
2
3
4
5
Galling index

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: Hb	0.82
Narrow-sense heritability: Hn	0.65
Number of effective factors: K1	0.30
Number of effective factors: K2	1.17

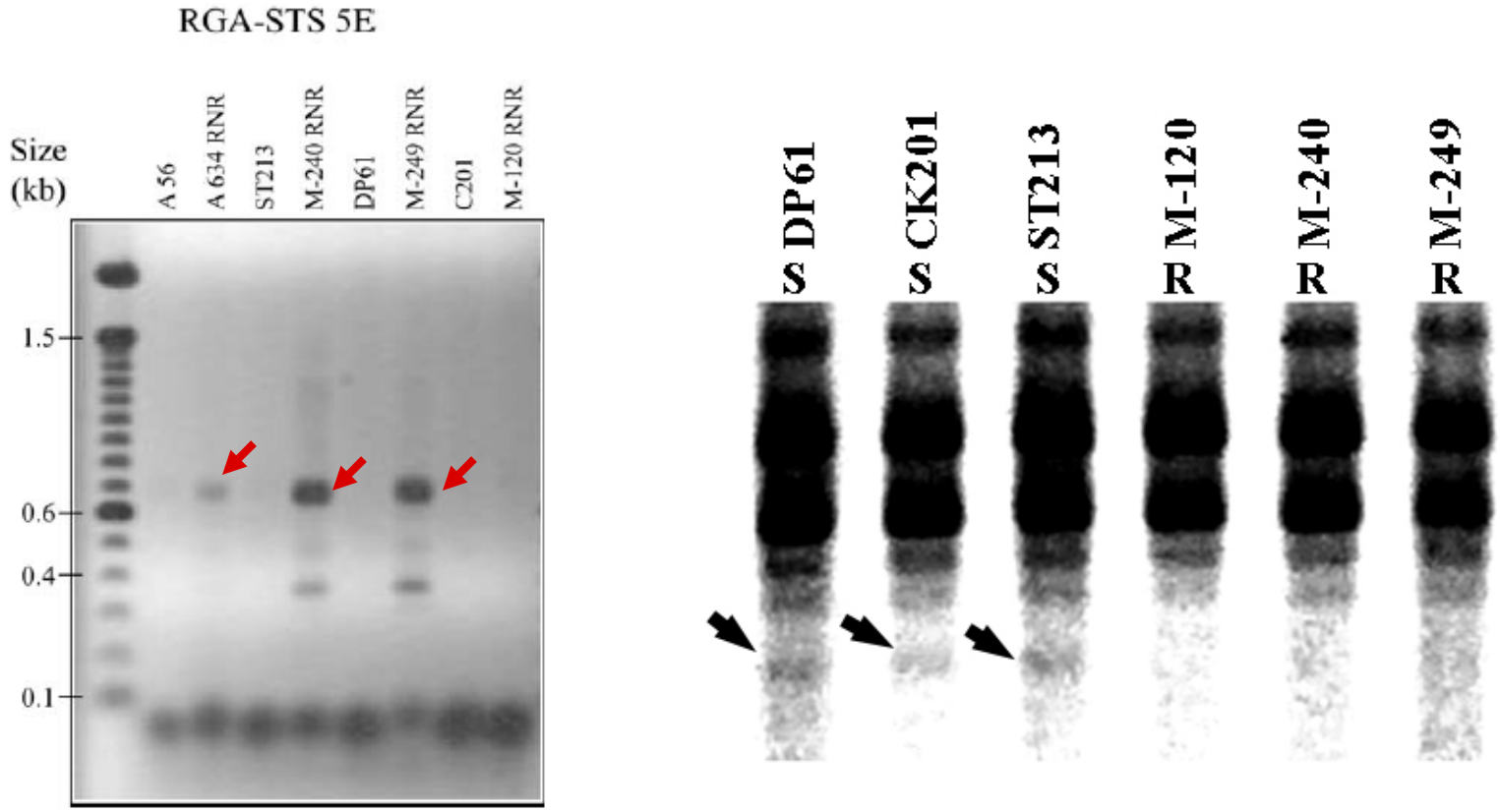
Segregation Data

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

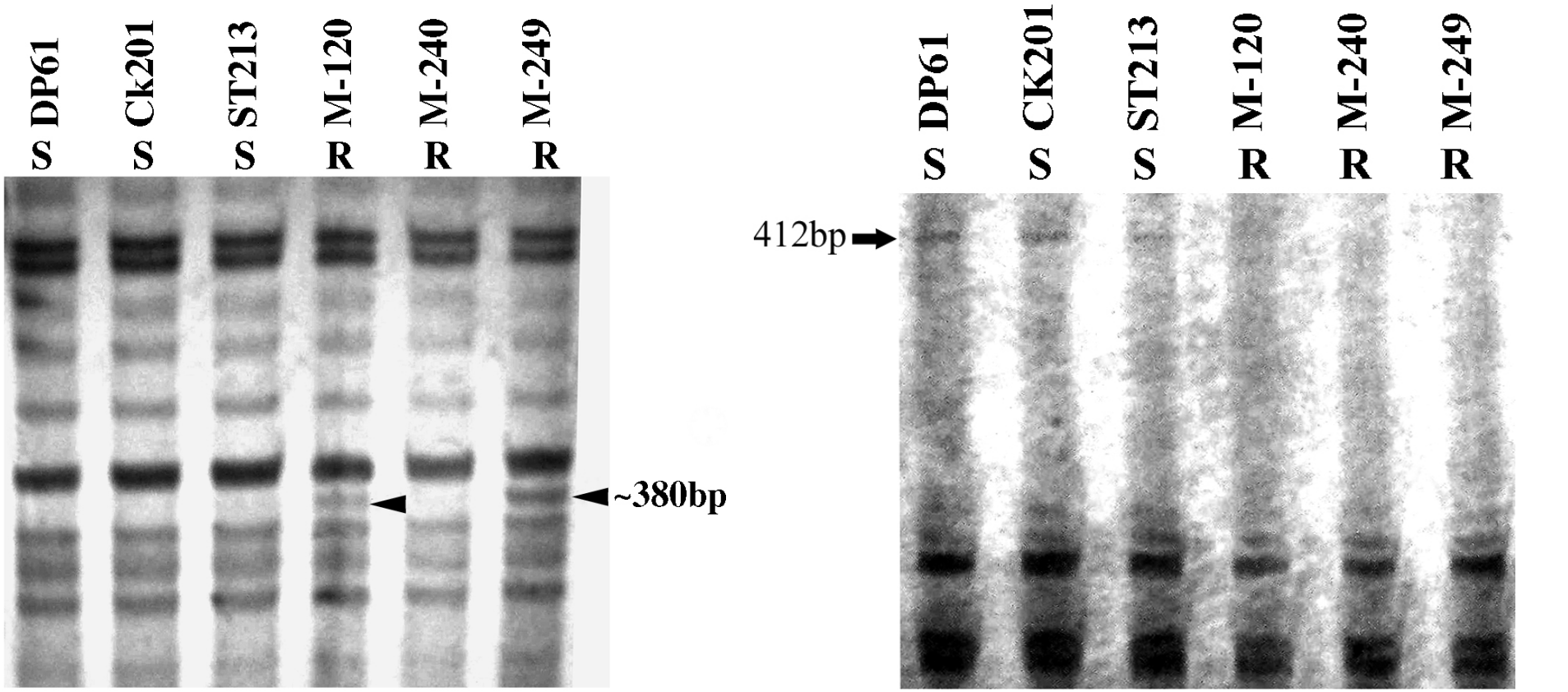
- **Auburn source: WM Jack Jones x Clevewilt ⟶ Auburn 623**
  - **Shepherd (1974): polygenic and partially dominant**
  - **Jenkins's lab (McPherson, 1993): 1 dominant & 1 additive**
  - **Starr's lab (Zhou, 1999): 2 dominant genes**
  - **McPherson et al. (2004): two genes, *Mi-1* & *Mi-2***
  - **G.h. 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
  - **Clevewilt: *Mi-2*?**
  - **Benzawada et al. (2003): 1 recessive gene**
  - **Zhou (1999): LA 887 (derived from Clevewilt) - 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

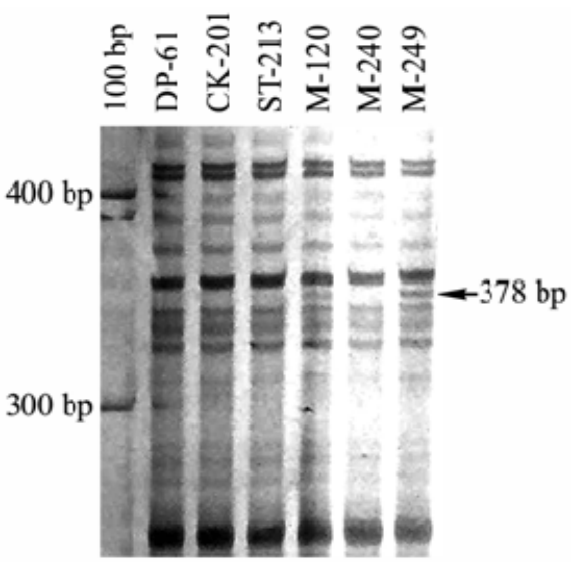


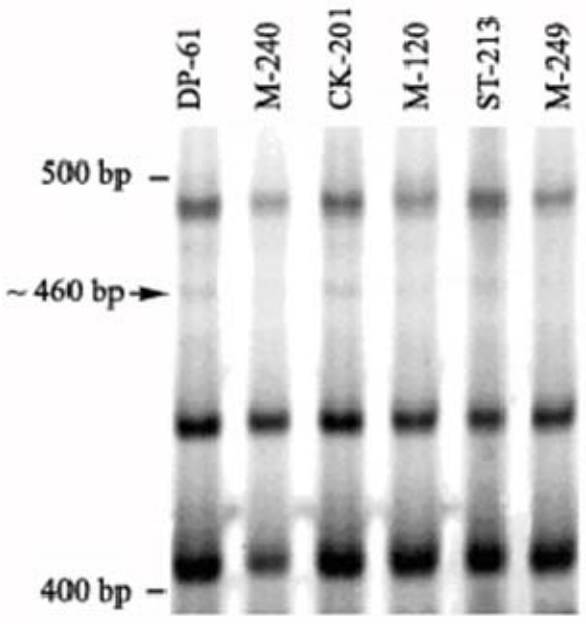
Genomic DNA from three pairs of NILs amplified with resistance gene analog (RGA) primers RGA-STS 5E and RLK.

Auburn 623 Source

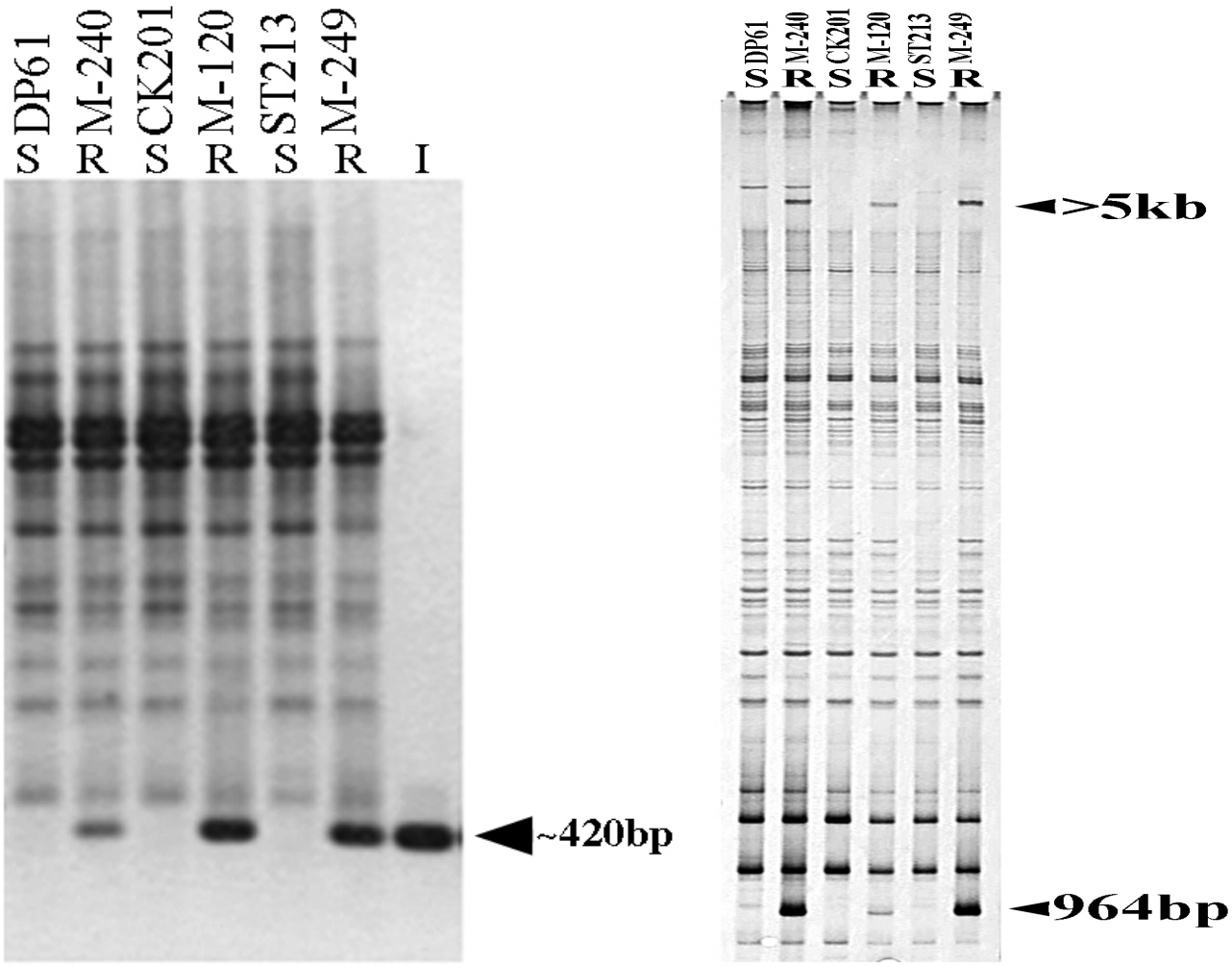


Two AFLP polymorphic markers amplified by primer pair B1 and C3 from genomic DNA isolated from near-isogenic lines resistant or susceptible to RKN.


100 bp
DP-61
CK-201
ST-213
M-120
M-240
M-249
400 bp
300 bp
378 bp

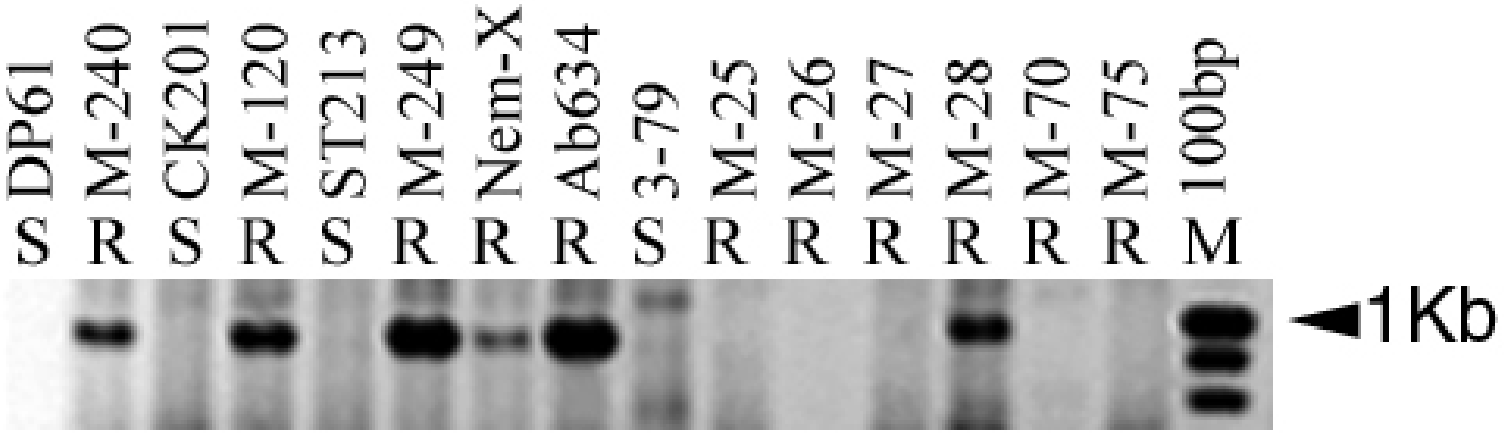

DP-61
M-240
CK-201
M-120
ST-213
M-249
500 bp
~460 bp
400 bp

Auburn 623 Source

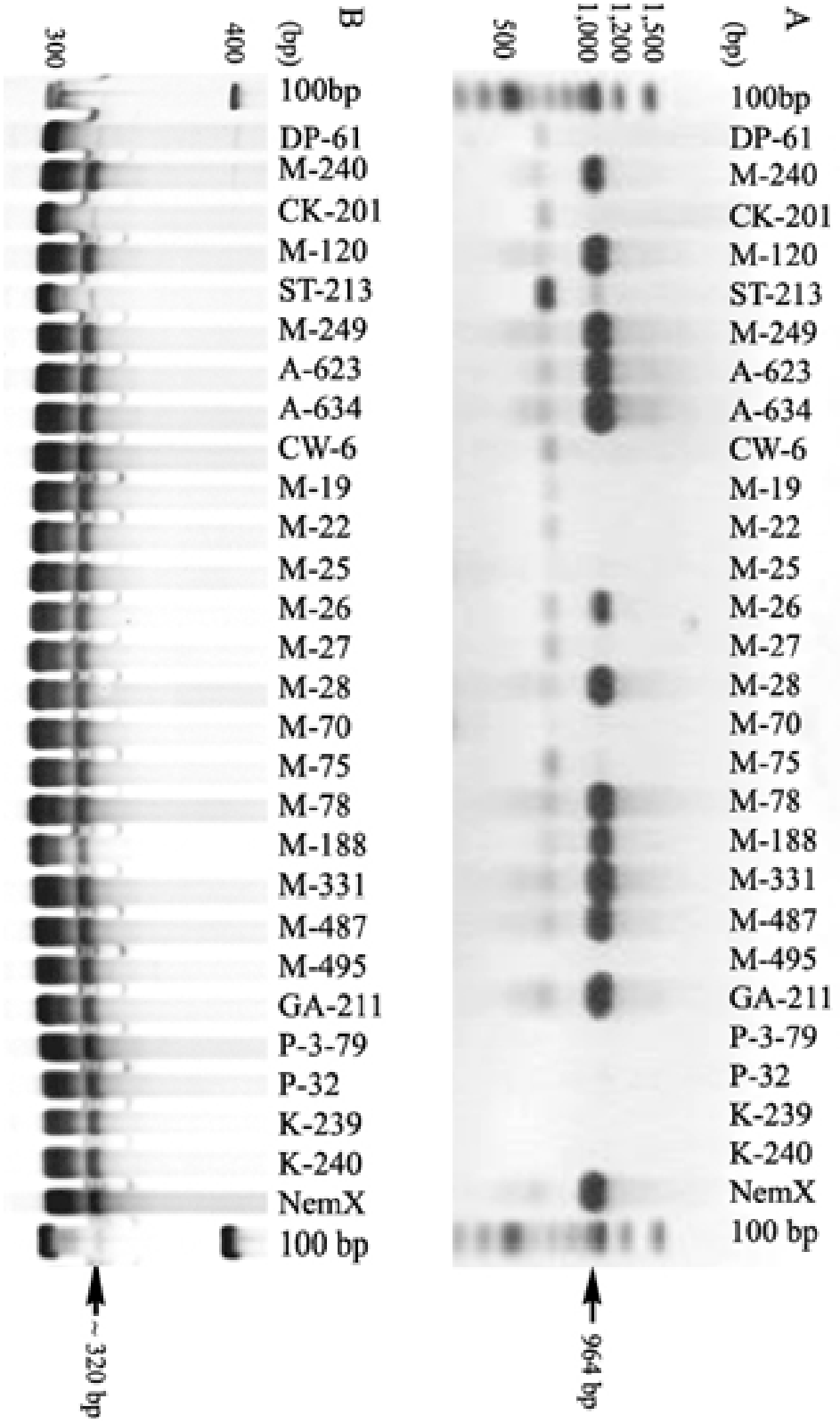


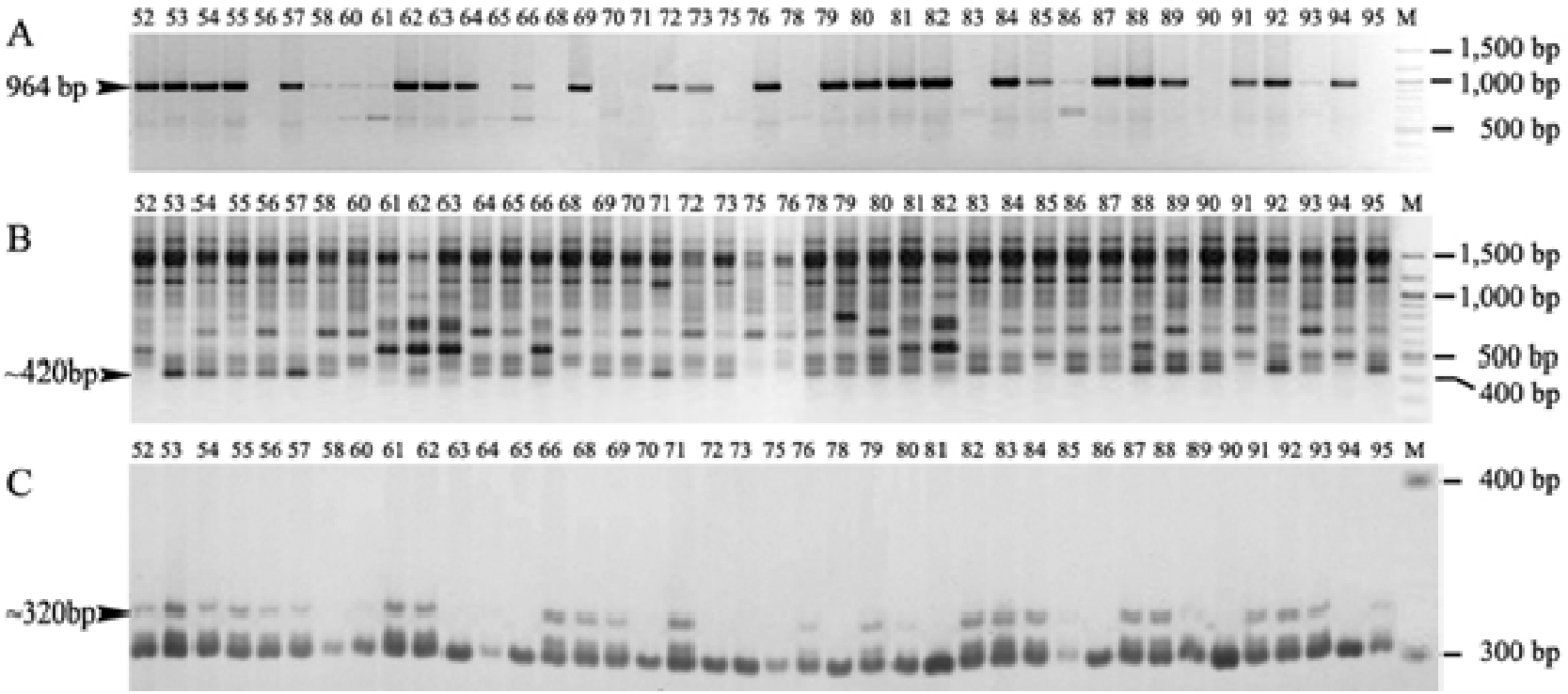
Three RAPD markers (NMRKN6931-420, NMRKN7811-1000, and NMRKN7811-5000) associated with cotton resistance to RKN.

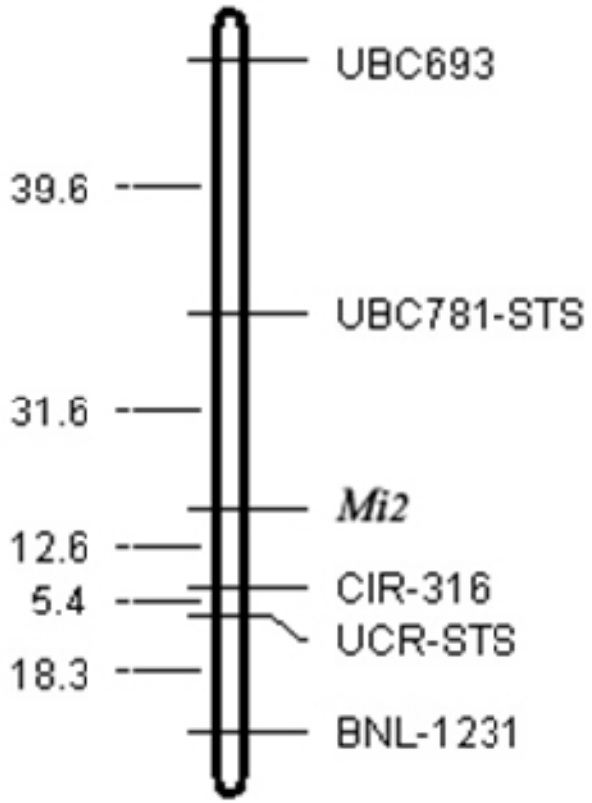
Auburn 623 Source



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.


B
(bp)
300
400
100bp
DP-61
M-240
CK-201
M-120
ST-213
M-249
A-623
A-634
CW-6
M-19
M-22
M-25
M-26
M-27
M-28
M-70
M-75
M-78
M-188
M-331
M-487
M-495
GA-211
P-3-79
P-32
K-239
K-240
NemX
100 bp
~ 320 bp
A
(bp)
500
1,000
1,200
1,500
100bp
DP-61
M-240
CK-201
M-120
ST-213
M-249
A-623
A-634
CW-6
M-19
M-22
M-25
M-26
M-27
M-28
M-70
M-75
M-78
M-188
M-331
M-487
M-495
GA-211
P-3-79
P-32
K-239
K-240
NemX
100 bp
964 bp


A
52 53 54 55 56 57 58 60 61 62 63 64 65 66 68 69 70 71 72 73 75 76 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 M
964 bp
1,500 bp
1,000 bp
500 bp
B
52 53 54 55 56 57 58 60 61 62 63 64 65 66 68 69 70 71 72 73 75 76 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 M
~420bp
1,500 bp
1,000 bp
500 bp
400 bp
C
52 53 54 55 56 57 58 60 61 62 63 64 65 66 68 69 70 71 72 73 75 76 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 M
~320bp
400 bp
300 bp


UBC693
39.6
UBC781-STS
31.6
Mi2
12.6
CIR-316
5.4
UCR-STS
18.3
BNL-1231

Germplasm Screening

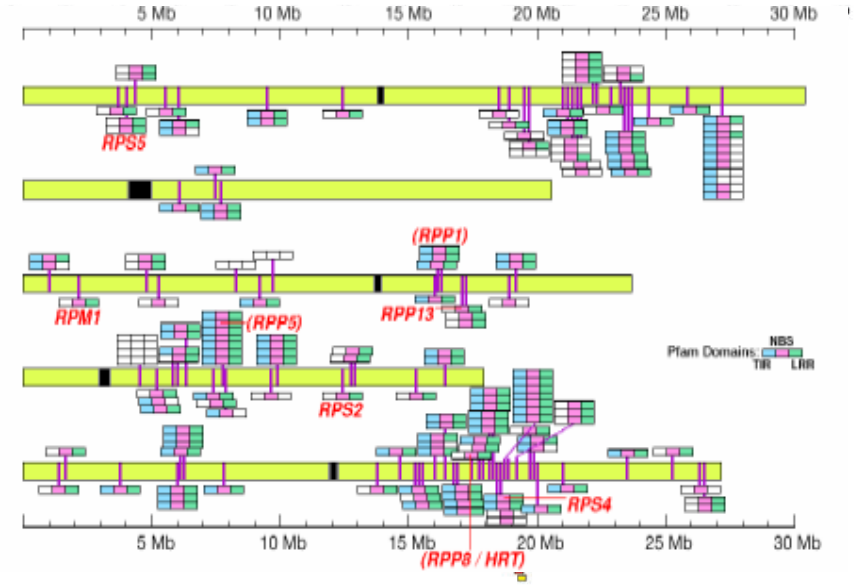
Genotype	PI No.	Pedigree	Reaction to RKN	UBC 693-420	UBC 781-1000	UBC 781-964 STS	UCR-STS	CIR 316	Group
DP 61			S	-	-	-	-	-	3
M-240	592511	AB 634 RNR / DP61	R	+	+	+	+	+	1
CK 201			S	-	-	-	-	-	3
M-120	592509	AB 634 RNR / CK201	R	+	+	+	+	+	1
ST 213			S	-	-	-	-	-	3
M-249	592512	AB 634 RNR / ST213	R	+	+	+	+	+	1
AB 623 RNR	SA 1492		R	+	+	+	+	+	1
AB 634 RNR	GP 166	AB 623 RNR / AB 56	R	+	+	+	+	+	1
Clevewilt 6	SA 245		?	-	-	-	+	+	3
M-19 RNR	517931	*Richmondii* / DP 16	R	+	-	-	+	+	2
M-22 RNR	517932	*Latifolium* / DP 16	R	+	-	-	+	-	2
M-25 RNR	517933	*Punctatum* / DP 16	R	+	-	-	+	-	2
M-26 RNR	517934	*Punctatum* / DP 16	R	+	-	-	+	-	2
M-27 RNR	517927	*Punctatum* / DP 16	R	+	-	-	+	-	2
M-28 RNR	517928	*Punctatum* / DP 16	R	+	+	+	+	-	1
M-70 RNR	517930	*Latifolium* / DP 16	R	+	-	-	+	+	2
M-75 RNR	517929	*Latifolium* / DP 16	R	+	-	-	+	+	2
M-78 RNR	517930	*Latifolium* / DP 16	R	+	+	+	+	+	1
M-188 RNR	517936	*Latifolium* / DP 16	R	+	+	+	+	-	1
M-331 RNR	592515	AB 634 RNR / AB56	R	+	+	+	+	+	1
M-487 RNR	517937	*Punctatum* / DP 16	R	+	+	+	+	+	1
M-495 RNR	517938	*Punctatum* / 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 Jack Jones	TX 2516		?	+	-	-	+	-	2
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)

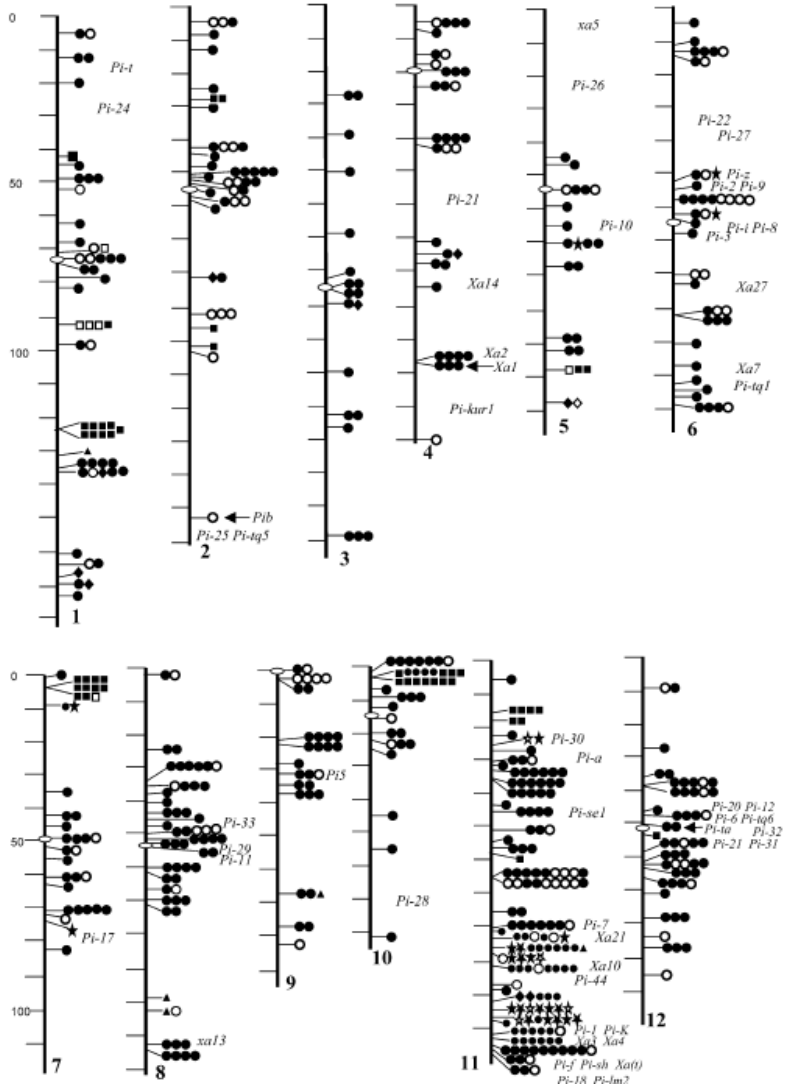
NBS-LRR Genes in Arabidopsis

(Meyers et al., 2003)

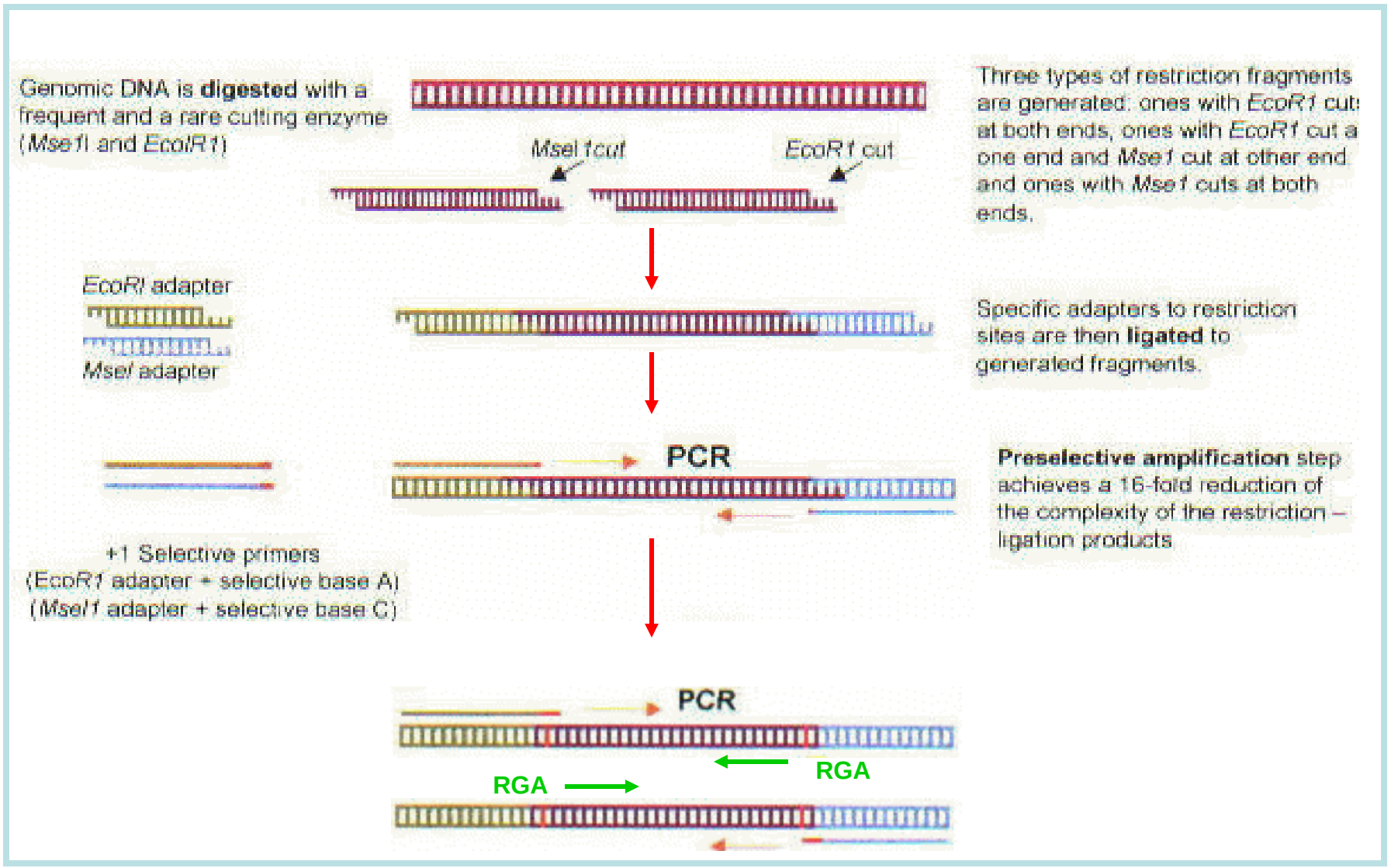


NBS Genes in Rice

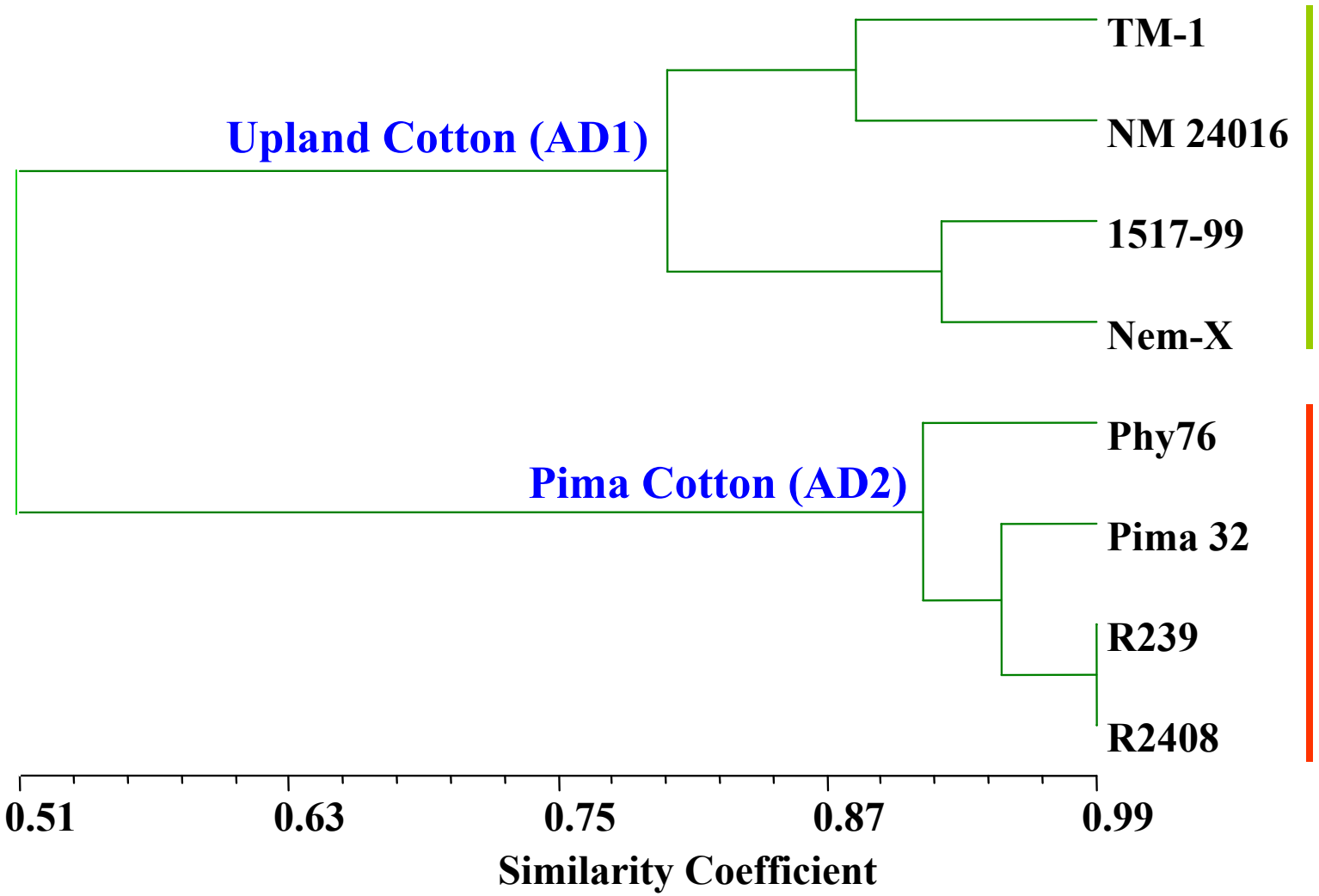
(Monosi et al., 2004)



RGA-AFLP Analysis

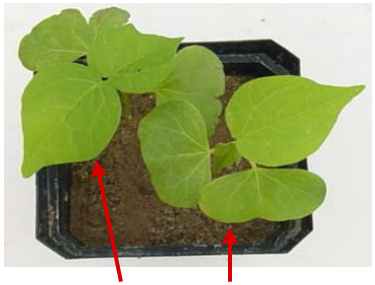


RGA-AFLP Analysis

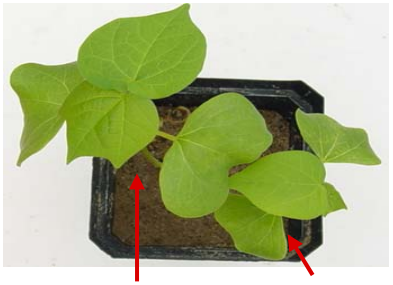


Cluster analysis based on RGA-AFLP

Root-knot Nematode Resistance



ST 474 1517-99 (CK)



Auburn 634 1517-99 (CK)

Chromosome 22

(29.0 %) 33.1
(20.9 %) 22.3
(40.5 %) 56.3
(26.9 %) 30.0

(15) BNL3065
(360) BNL1231-180
(17) BNL3279
(56) BNL1681-2
(265) RLKF-3-450w

Chromosome 11

Dis (cM) Markers

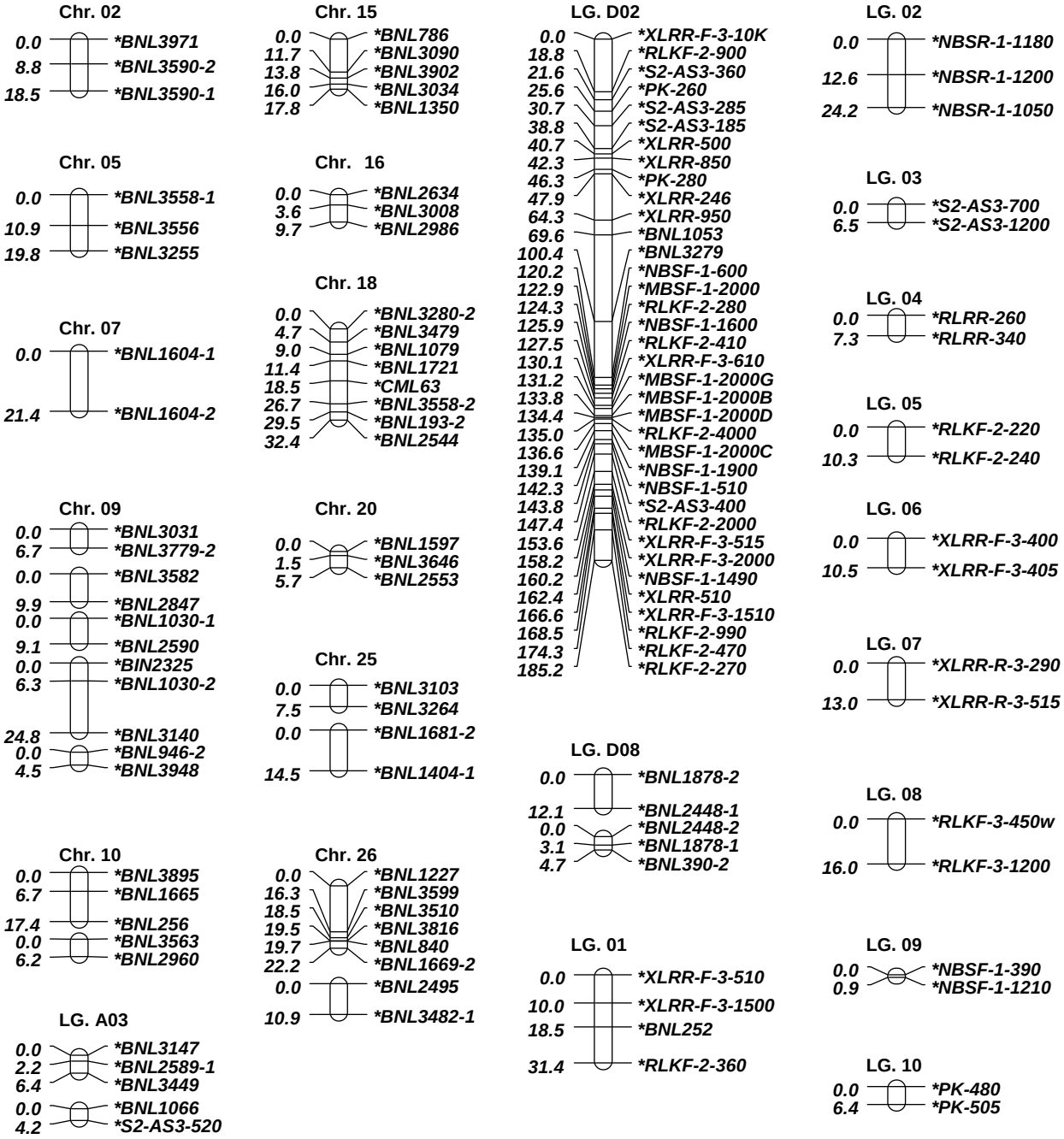
BNL1231
14.0
rkn1
2.6
M1-327 → AFLP
0.9
CIR316a GHACC1 → UCRSTS
5.8
M2-545
3.8
M3-258
1.1
M3-257

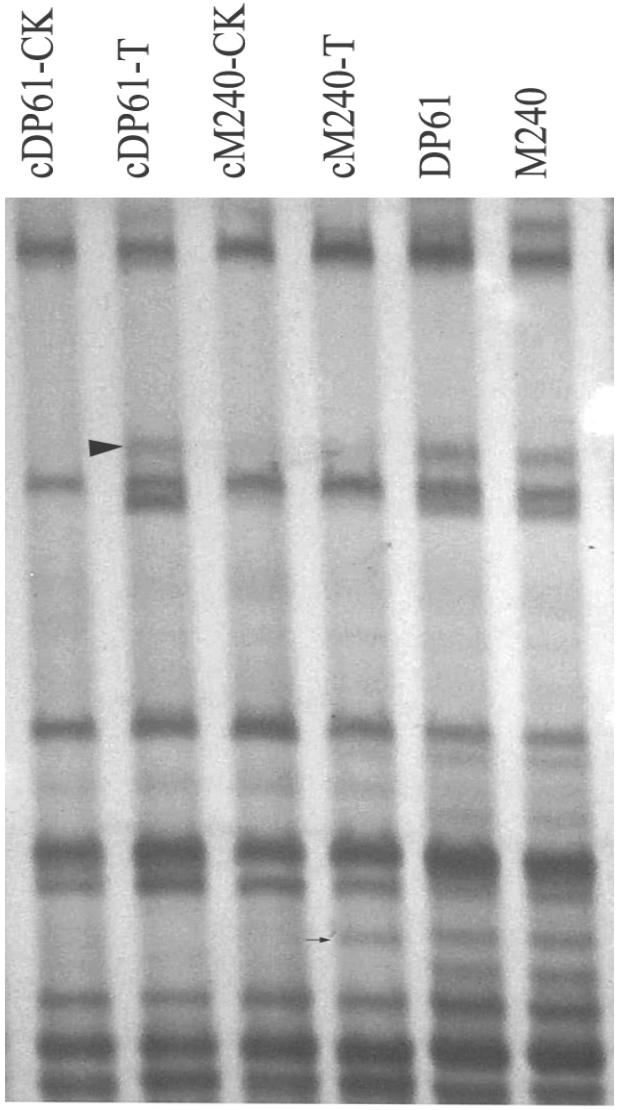
Wang & Roberts (2006)

Chromosome 11

(30.3 %) 35.1
(6.3 %) 6.3
(23.5 %) 25.5
(19.5 %) 20.1
(39.6 %) 53.9
(7.1 %) 7.2
(15.2 %) 15.7

(34) BNL3649
(172) S2-AS3-520
(96) BNL1066
(355) UCRSTS-305
(356) CIR316-221R
(292) XLRR-R-3-1050
(287) XLRR-R-3-620
(4) BNL3442


Chr. 02
0.0 *BNL3971
8.8 *BNL3590-2
18.5 *BNL3590-1
Chr. 05
0.0 *BNL3558-1
10.9 *BNL3556
19.8 *BNL3255
Chr. 07
0.0 *BNL1604-1
21.4 *BNL1604-2
Chr. 09
0.0 *BNL3031
6.7 *BNL3779-2
0.0 *BNL3582
9.9 *BNL2847
0.0 *BNL1030-1
9.1 *BNL2590
0.0 *BIN2325
6.3 *BNL1030-2
24.8 *BNL3140
0.0 *BNL946-2
4.5 *BNL3948
Chr. 10
0.0 *BNL3895
6.7 *BNL1665
17.4 *BNL256
0.0 *BNL3563
6.2 *BNL2960
LG. A03
0.0 *BNL3147
2.2 *BNL2589-1
6.4 *BNL3449
0.0 *BNL1066
4.2 *S2-AS3-520
Chr. 15
0.0 *BNL786
11.7 *BNL3090
13.8 *BNL3902
16.0 *BNL3034
17.8 *BNL1350
Chr. 16
0.0 *BNL2634
3.6 *BNL3008
9.7 *BNL2986
Chr. 18
0.0 *BNL3280-2
4.7 *BNL3479
9.0 *BNL1079
11.4 *BNL1721
18.5 *CML63
26.7 *BNL3558-2
29.5 *BNL193-2
32.4 *BNL2544
Chr. 20
0.0 *BNL1597
1.5 *BNL3646
5.7 *BNL2553
Chr. 25
0.0 *BNL3103
7.5 *BNL3264
0.0 *BNL1681-2
14.5 *BNL1404-1
Chr. 26
0.0 *BNL1227
16.3 *BNL3599
18.5 *BNL3510
19.5 *BNL3816
19.7 *BNL840
22.2 *BNL1669-2
0.0 *BNL2495
10.9 *BNL3482-1
LG. D02
0.0 *XLRR-F-3-10K
18.8 *RLKF-2-900
21.6 *S2-AS3-360
25.6 *PK-260
30.7 *S2-AS3-285
38.8 *S2-AS3-185
40.7 *XLRR-500
42.3 *XLRR-850
46.3 *PK-280
47.9 *XLRR-246
64.3 *XLRR-950
69.6 *BNL1053
100.4 *BNL3279
120.2 *NBSF-1-600
122.9 *MBSF-1-2000
124.3 *RLKF-2-280
125.9 *NBSF-1-1600
127.5 *RLKF-2-410
130.1 *XLRR-F-3-610
131.2 *MBSF-1-2000G
133.8 *MBSF-1-2000B
134.4 *MBSF-1-2000D
135.0 *RLKF-2-4000
136.6 *MBSF-1-2000C
139.1 *NBSF-1-1900
142.3 *NBSF-1-510
143.8 *S2-AS3-400
147.4 *RLKF-2-2000
153.6 *XLRR-F-3-515
158.2 *XLRR-F-3-2000
160.2 *NBSF-1-1490
162.4 *XLRR-510
166.6 *XLRR-F-3-1510
168.5 *RLKF-2-990
174.3 *RLKF-2-470
185.2 *RLKF-2-270
LG. D08
0.0 *BNL1878-2
12.1 *BNL2448-1
0.0 *BNL2448-2
3.1 *BNL1878-1
4.7 *BNL390-2
LG. 01
0.0 *XLRR-F-3-510
10.0 *XLRR-F-3-1500
18.5 *BNL252
31.4 *RLKF-2-360
LG. 02
0.0 *NBSR-1-1180
12.6 *NBSR-1-1200
24.2 *NBSR-1-1050
LG. 03
0.0 *S2-AS3-700
6.5 *S2-AS3-1200
LG. 04
0.0 *RLRR-260
7.3 *RLRR-340
LG. 05
0.0 *RLKF-2-220
10.3 *RLKF-2-240
LG. 06
0.0 *XLRR-F-3-400
10.5 *XLRR-F-3-405
LG. 07
0.0 *XLRR-R-3-290
13.0 *XLRR-R-3-515
LG. 08
0.0 *RLKF-3-450w
16.0 *RLKF-3-1200
LG. 09
0.0 *NBSF-1-390
0.9 *NBSF-1-1210
LG. 10
0.0 *PK-480
6.4 *PK-505



cDNA-AFLP analysis

Marker Screening

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