

Mapping Resistance to Root-knot Nematodes



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Root-Knot Nematode (*M. incognita*)

Major Economic Pest in Cotton



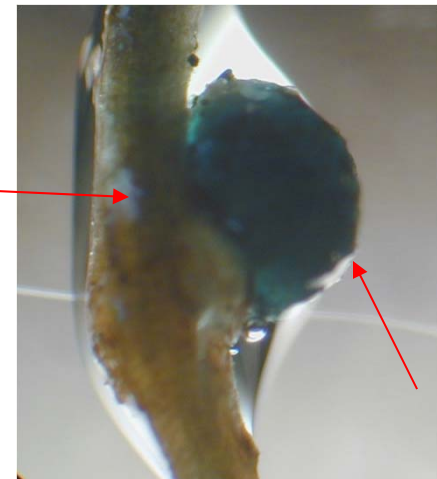
Res



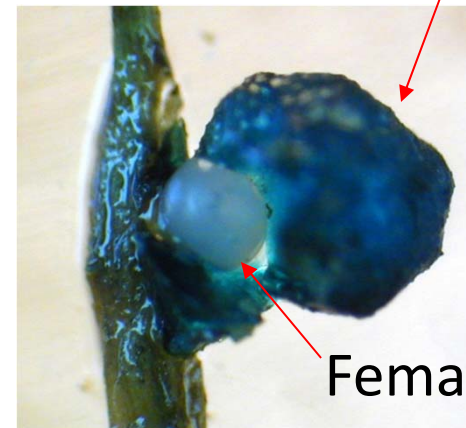
Sus



J₂



Egg Mass

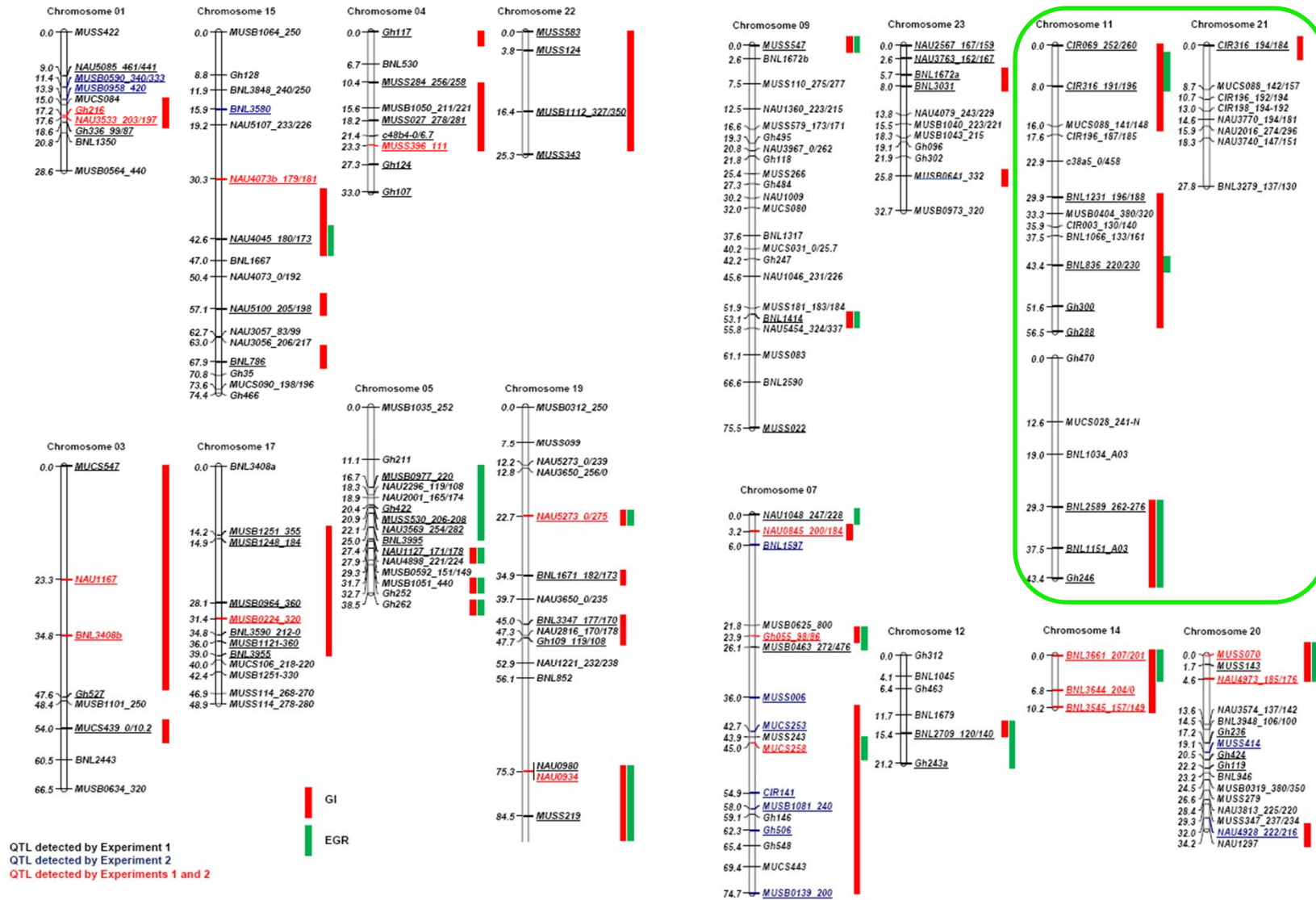


Female

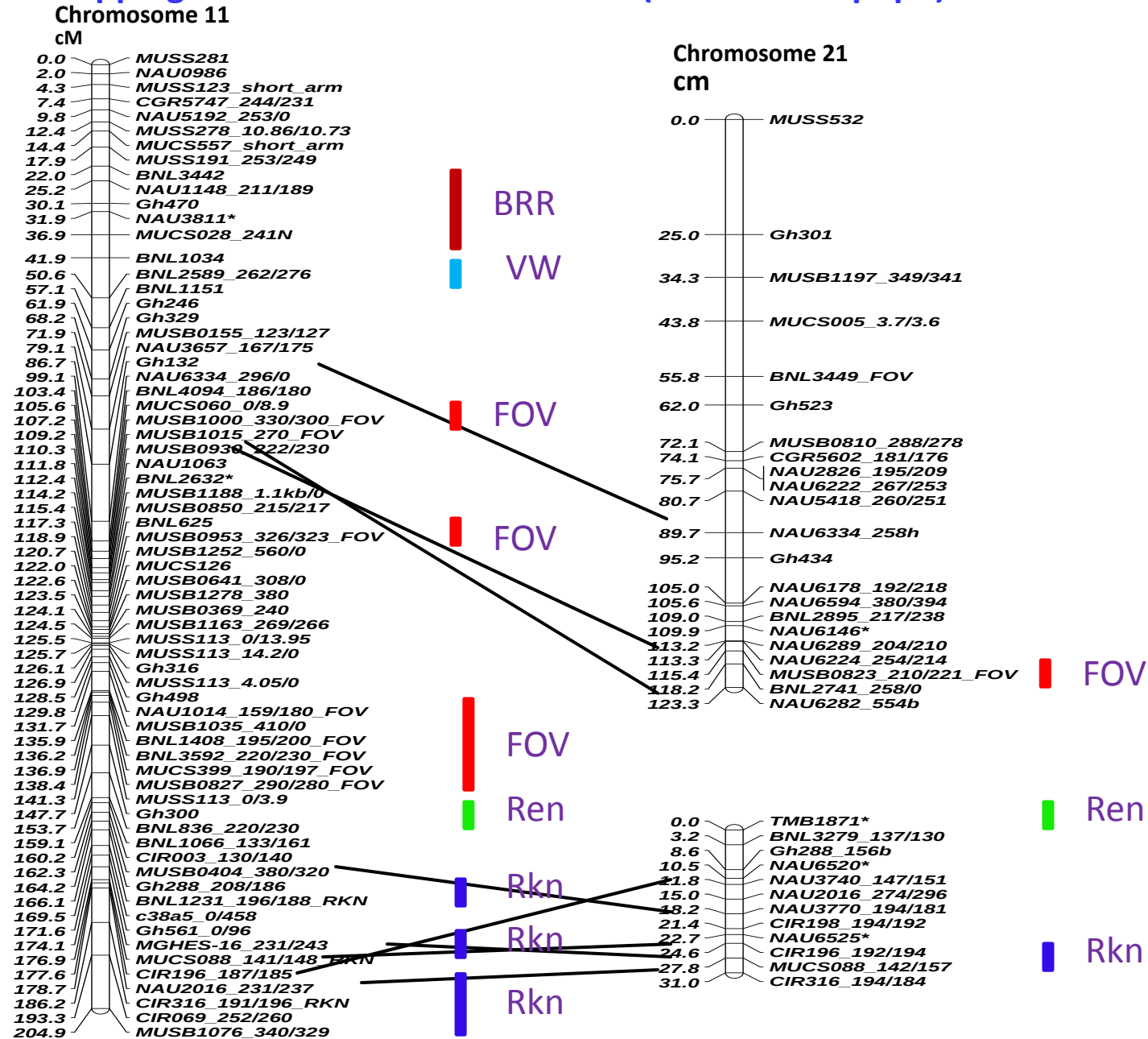
Res X Sus Transgressive Resistance
Intraspecific cross NemX x SJ-2
Interspecific cross Pima S-7 x NemX

Sus x Sus Transgressive Resistance
Interspecific Cross TM1 x Pima 3-79

Map locations of QTLs associated with reaction to root-knot nematode in TM-1 x Pima 3-79



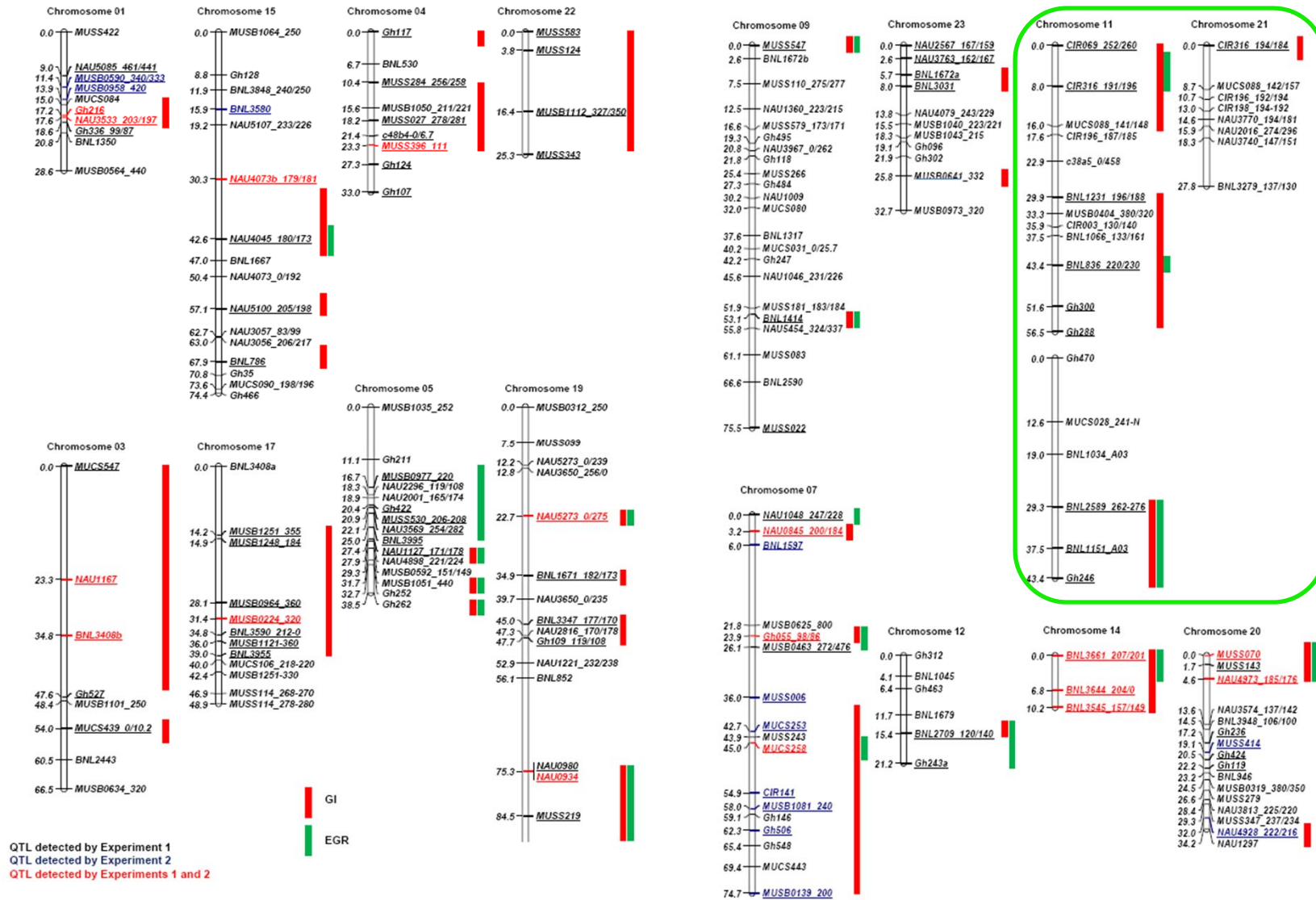
Mapping BACs onto Chr11 and Chr21 (TM-1 x 3-79 popn)



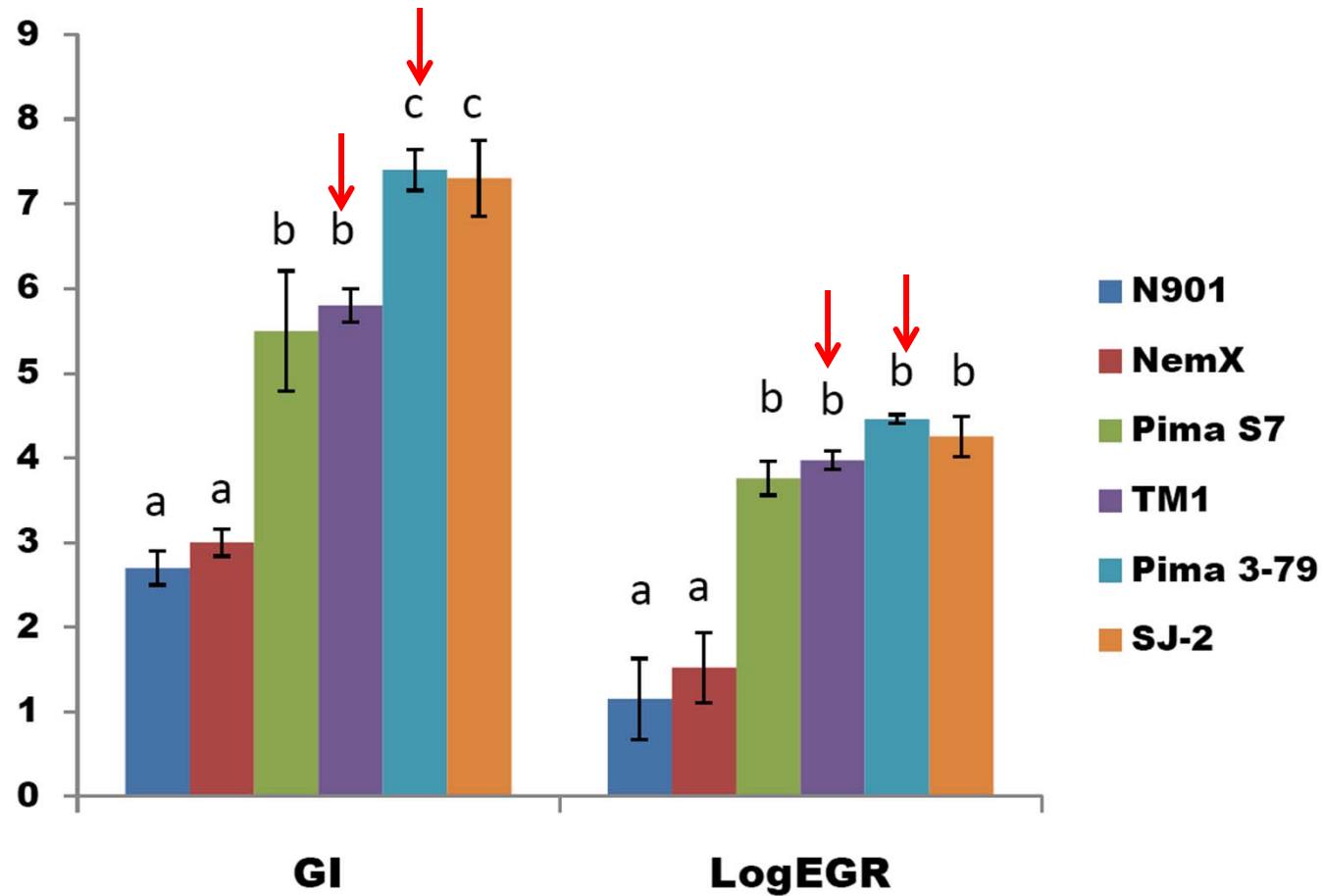
TM-1 x Pima 3-79 is a standard population used for genetic and genomic analysis on cotton



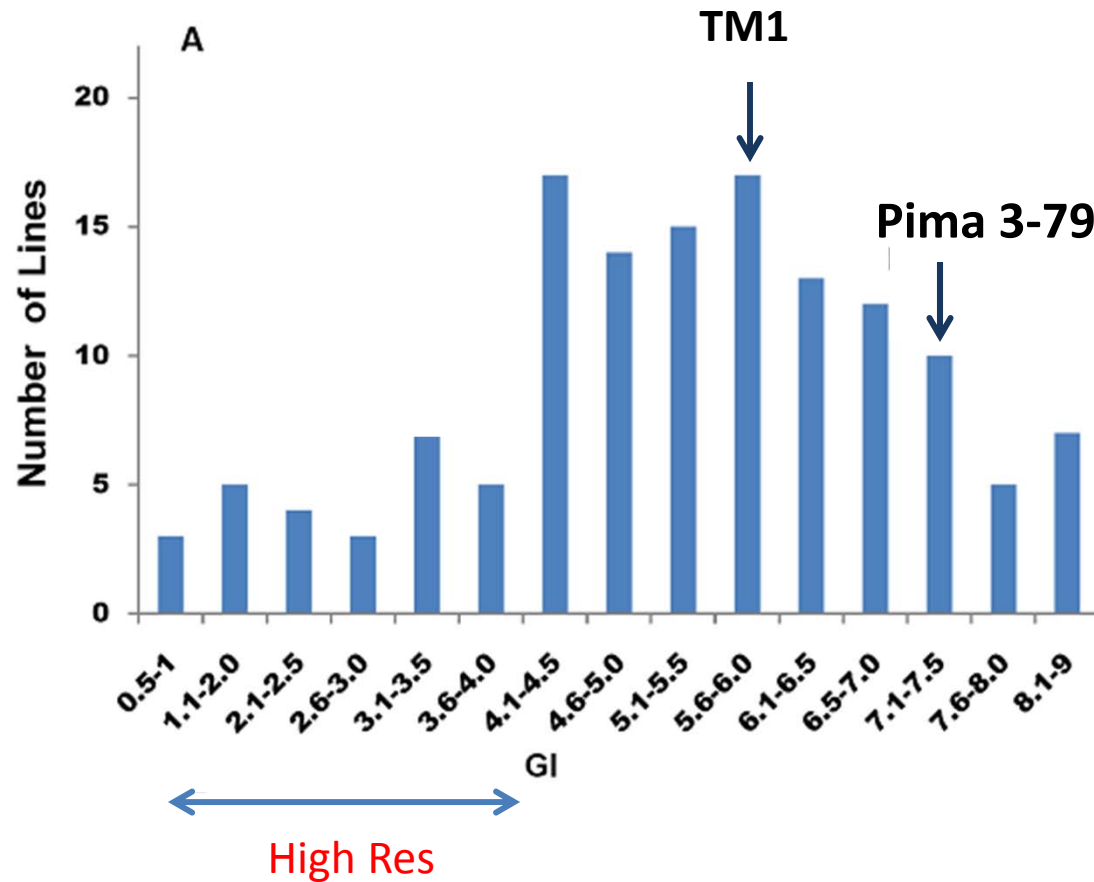
Map locations of QTLs associated with reaction to root-knot nematode in TM-1 x Pima 3-79



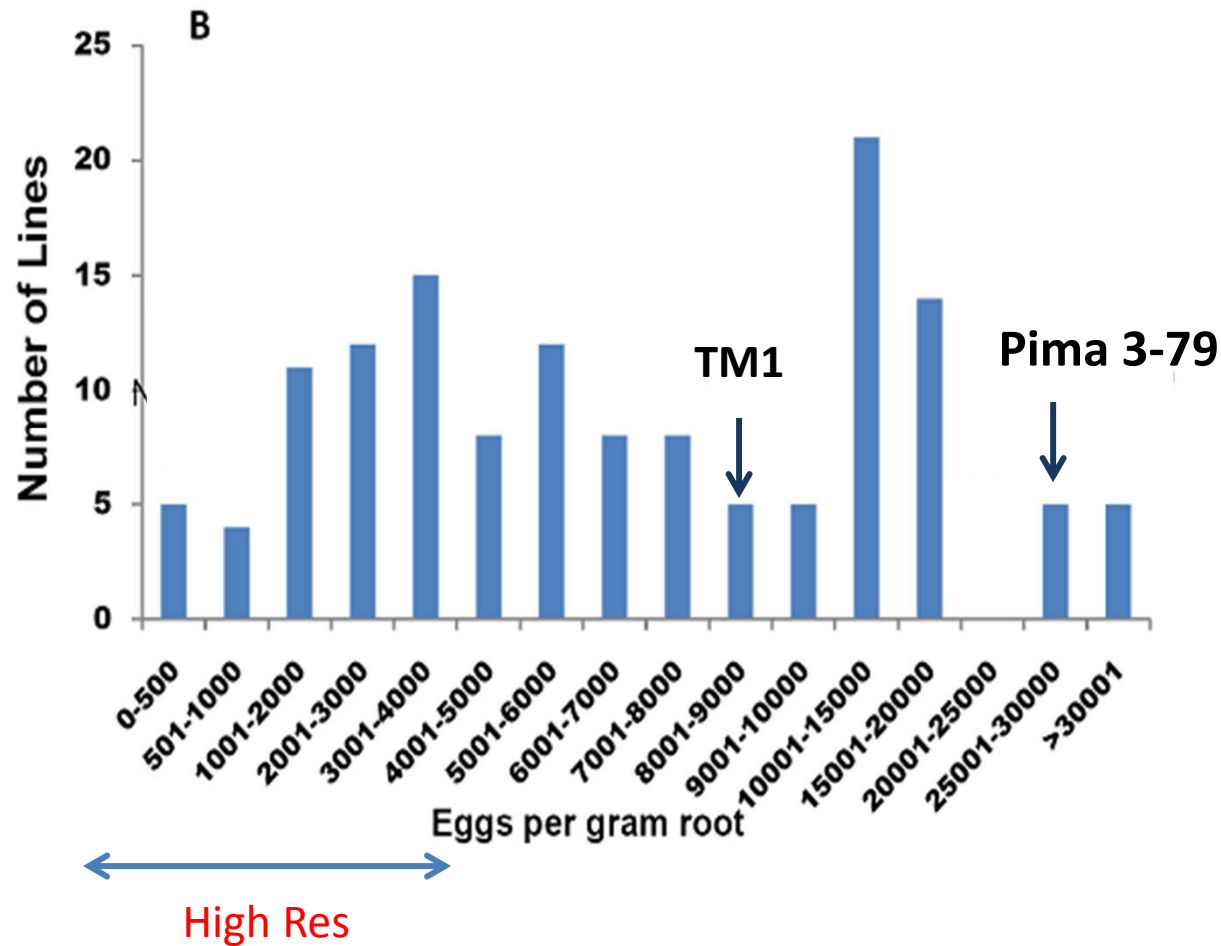
Parental reaction to root-knot nematodes



Extreme phenotypes of transgressive segregants outside parent range in RIL TM1 x Pima 3-79



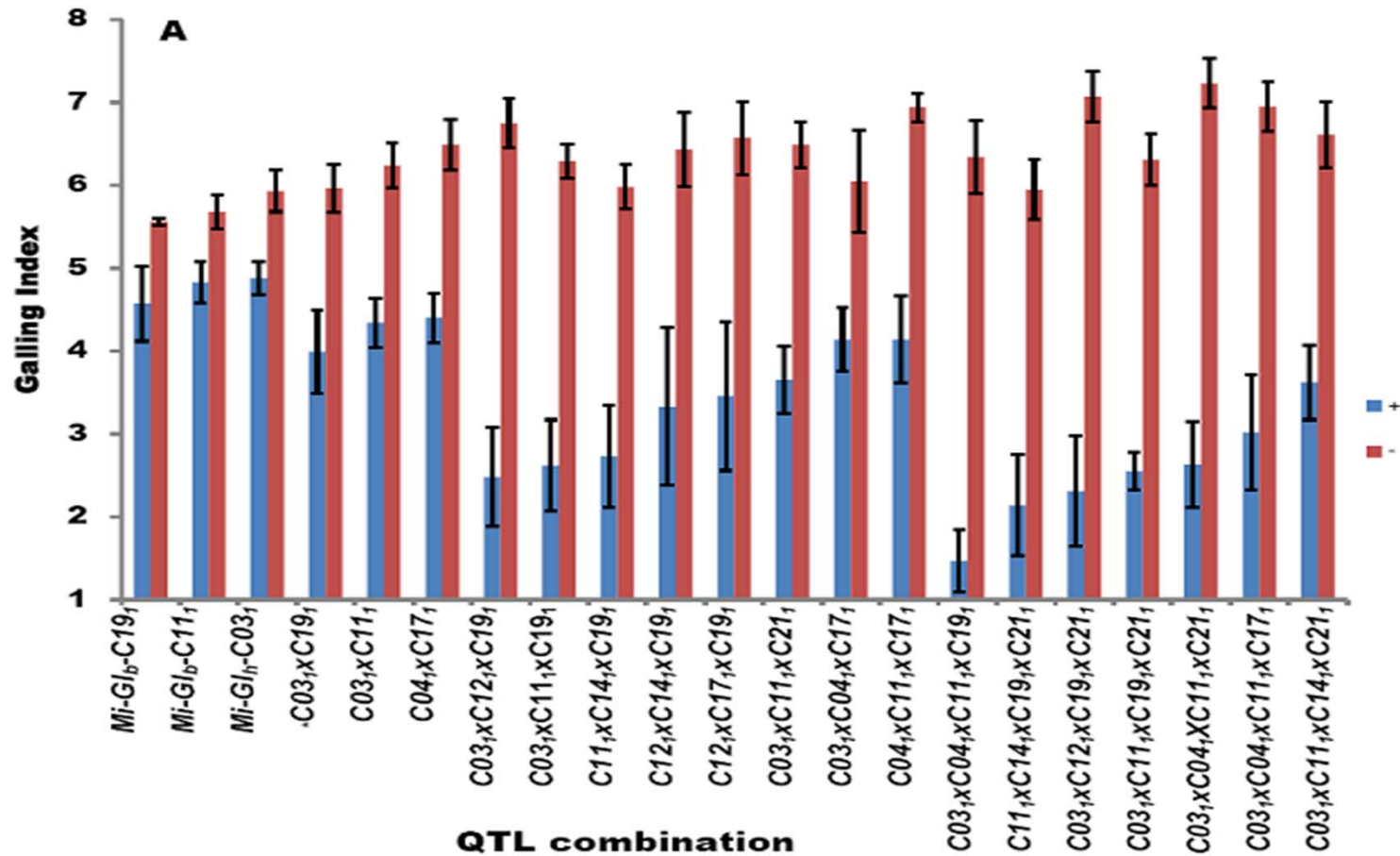
Extreme phenotypes of transgressive segregants outside parent range in RIL TM1 x Pima 3-79



QTLs associated with galling index and egg production

QTL-GI ^a	Name	Chr ^c	Locus	K ^{*e}	Signif. ^f	from TM-1 allele ^g	From Pima 3-79 allele
1	<i>Mi-GI_h-C03₁</i> ^d	3	BNL3408b	12.32	*****	Yes	
2	<i>Mi-GI_h-C17₁</i>	17	MUSB0224/320	11.534	*****	Yes	
3	<i>Mi-GI_b-C04₁</i>	4	MUSS396_111	10.071	*****		Yes
4	<i>Mi-GI_b-C11₁</i>	11	BNL1231b_196/188	7.95	*****		Yes
5	<i>Mi-GI_b-C22₁</i>	22	MUSB1112_327/350	7.744	***		Yes
6	<i>Mi-GI_b-C05₁</i>	5	Gh262	7.493	***		Yes
7	<i>Mi-GI_h-C15₁</i>	15	NAU4045_170/165	7.34	***	Yes	
8	<i>Mi-GI_h-C14₁</i>	14	BNL3661_207/201	7.103	***	Yes	
9	<i>Mi-GI_b-C20₁</i>	20	Gh119	6.914	***		Yes
10	<i>Mi-GI_h-C19₁</i>	19	MUSS219	6.872	***	Yes	
11	<i>Mi-GI_h-C07₁</i>	7	Gh055_98/86	6.315	**	Yes	
12	<i>Mi-GI_b-C12₁</i>	12	MUSB0117_123/143	6.134	**		Yes
13	<i>Mi-GI_b-C21₁</i>	21	CIR316_194/184	4.041	**		Yes
14	<i>Mi-GI_b-C19₁</i>	19	NAU5273_0/275	4.197	**		Yes
QTL- EGR ^b							
1	<i>Mi-EGR_b-C23₁</i>	23	BNL1672a	10.644	*****		Yes
2	<i>Mi-EGR_h-C14₁</i>	14	BNL3661_207/201	9.705	*****	Yes	
3	<i>Mi-EGR_h-C24₁</i>	24	NAU3605_222/227	7.279	***	Yes	
4	<i>Mi-EGR_h-C06₁</i>	6	NAU4969_0/305	7.253	***	Yes	
5	<i>Mi-EGR_h-C15₁</i>	15	NAU4045_180/173	6.244	**	Yes	
6	<i>Mi-EGR_b-C11₁</i>	11	CIR316_191/196	5.32	**		Yes
7	<i>Mi-EGR_h-C19₁</i>	19	MUSS219	5.448	**	Yes	
8	<i>Mi-EGR_h-C12₁</i>	12	Gh243a	4.862	**	Yes	
9	<i>Mi-EGR_b-C11₂</i>	11	BNL2589_262/276	4.778	**		Yes

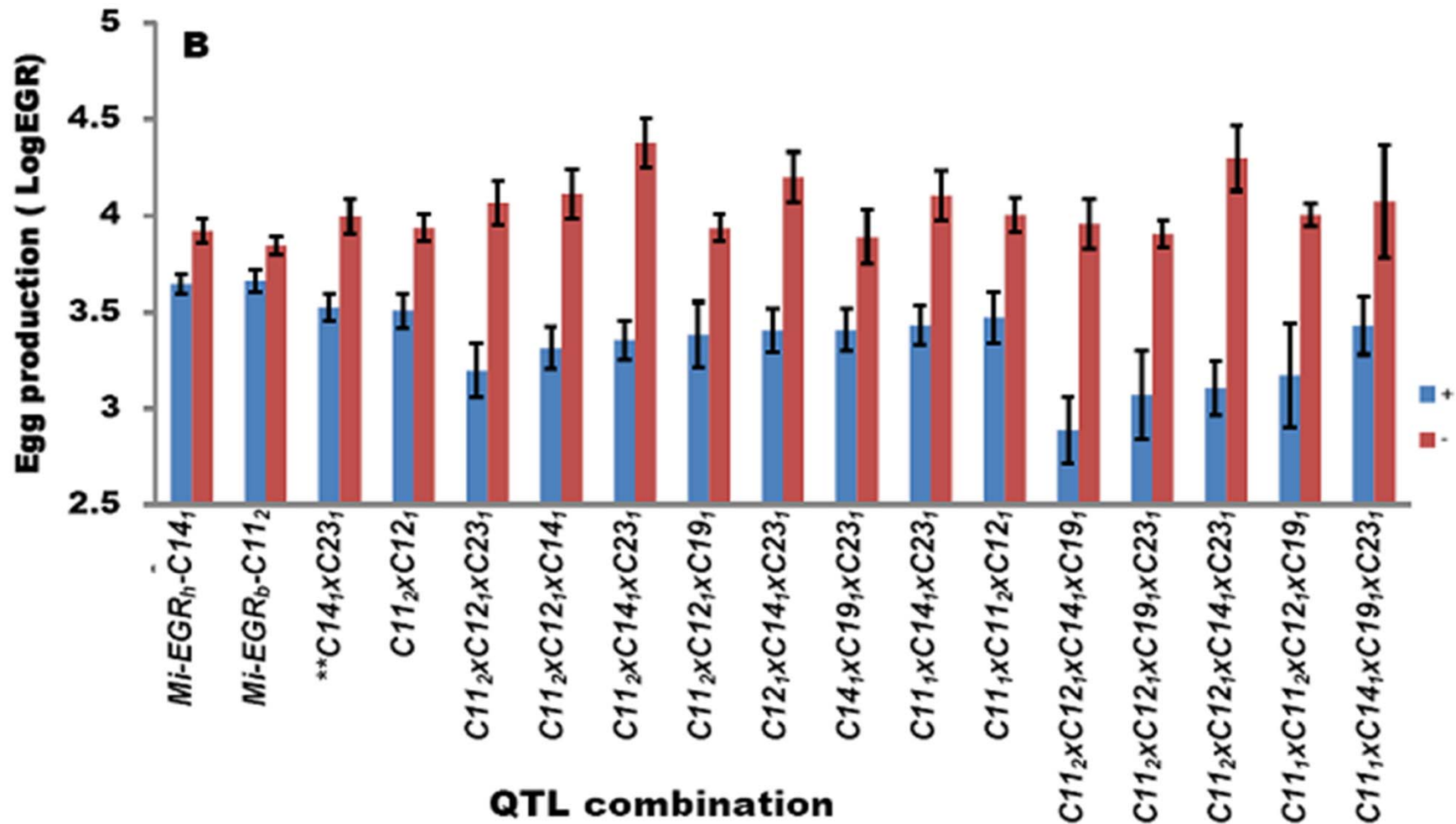
Comparison of lines with/without combinations of 2 to 4 QTLs showed reduction > 50% in both Gallling Index (GI) and Egg production



C03 x C04 x C11 x C19 GI 1.7

TM1 x P3-79 x P3-79 x TM1

Combinations of 2 to 4 QTLs
reduced egg production > 50%



F7 (Pima S-7 x NemX) Chr 11 Gallig Index

MapQTL5-Interval Mapping

Position	Locus	LOD	# Iter.	mu_A	mu_B	% Expl.	Additive
0	HAU1809-283/275	10.57	5	4.01609	1.77343	29.8	1.12133
1.94	BNL2650-222/0	8.64	5	4.01487	1.93571	25.1	1.03958
4.598	CIR112-251/260	10.79	6	4.13271	1.83271	30.8	1.15
6.345	HAU1283-149/155	13.67	6	4.23021	1.70643	37.4	1.26189
7.237	DC40316-348/344	12.48	6	4.15922	1.74488	34.3	1.20717
9.538	BNL2650-0/229	10.09	5	4.11101	1.89044	28.5	1.11029
11.981	CIR069-271/0	12.25	5	3.96229	1.60458	33.3	1.17886
14.775	CIR069-0/267	15.39	5	4.28203	1.67539	39.8	1.30332
18.458	MGHES-16-255/256	8.77	5	4.00308	1.91788	25.3	1.0426
19.381	CIR316-215/0	14.12	5	4.23251	1.71262	37.2	1.25995
21.004	CIR316-0/222	15.09	5	4.18672	1.61852	39.2	1.2841
21.891	MUSB1076-296/306	14.32	5	4.1286	1.61612	37.6	1.25624
26.242	NAU5428-236/0	7.35	4	3.75378	1.85909	21.5	0.947347
26.621	HAU2681-366/0	8.11	4	3.80151	1.82292	23.4	0.989295
27.205	CM140-0/125	7.5	4	3.79082	1.87678	21.9	0.957022
27.434	CM140-117/0	8.9	4	3.85266	1.79173	25.4	1.03046
27.531	NAU3770-187/195	9.14	4	3.88029	1.79459	26	1.04285
27.657	MUCS088-170/0	11.16	4	3.93949	1.67301	30.7	1.13324
28.397	NAU1232-205/208	7.92	5	3.82405	1.84483	23.4	0.989614
28.616	CIR196-205/206	10.32	5	3.94257	1.73395	29.1	1.10431
29.315	MUCS088-0/162	9.71	4	3.91343	1.77194	27.4	1.07074
29.846	MGHES-16-0/221	9.56	5	3.96716	1.8314	27	1.06788
30.165	NAU2016-0/247	9.59	5	3.95194	1.81604	27.1	1.06795
32.482	NAU3390-213/206	6.32	5	3.80069	2.01338	18.8	0.893655
33.065	BNL1231-208/217	6.06	5	3.77474	2.01437	18.3	0.880185
33.353	NAU2152-221/215	5.81	5	3.77281	2.04819	17.5	0.862309
35.642	Gh288-173/189	5.19	5	3.62729	1.99738	15.9	0.814957
36.435	MUSB0404-320/328	4.48	5	3.56811	2.04451	13.9	0.761803
37.011	CIR003-149/147	3.67	4	3.53343	2.14864	11.4	0.692396
39.594	BNL4011-175/0	2.15	5	3.32099	2.23839	7	0.541297
56.09	MUSB0404-0/314	1.12	4	3.19168	2.41131	3.6	0.390186
57.238	NAU5428-0/234	2.83	5	4.04226	2.49035	9	0.775955

Chr 11 Egg production Log(EGR+1)

MapQTL5-Interval Mapping

Position	Locus	LOD	mu_A	mu_B	Variance	% Expl.	Additive
0	HAU1809-283/275	11.24	2.61385	0.978945	1.45543	31.3	0.817454
1.94	BNL2650-222/0	12.38	2.72567	1.01573	1.40836	33.5	0.854966
4.598	CIR112-251/260	15.74	2.81788	0.925417	1.24435	41.3	0.946231
6.345	HAU1283-149/155	21.06	2.9234	0.819199	1.03274	51.3	1.0521
7.237	DC40316-348/344	18.84	2.85103	0.850989	1.13411	46.5	1.00002
9.538	BNL2650-0/229	14.89	2.81673	0.97333	1.29733	38.8	0.921699
11.981	CIR069-271/0	13.76	2.6052	0.839933	1.33983	36.8	0.882635
14.775	CIR069-0/267	15.3	2.78138	0.931534	1.27994	39.6	0.924923
18.458	MGHES-16-255/256	6.39	2.48102	1.18275	1.70826	19.4	0.649131
19.381	CIR316-215/0	9.39	2.59228	1.07624	1.55553	26.6	0.758018
21.004	CIR316-0/222	13.76	2.6828	0.91977	1.34712	36.4	0.881516
21.891	MUSB1076-296/306	12.55	2.6322	0.926475	1.39414	34.2	0.852861
26.242	NAU5428-236/0	5.65	2.33558	1.13684	1.75954	17	0.59937
26.621	HAU2681-366/0	5.93	2.3523	1.12583	1.74278	17.7	0.613236
27.205	CM140-0/125	4.66	2.30029	1.20186	1.81762	14.2	0.549211
27.434	CM140-117/0	6.62	2.38978	1.10185	1.70435	19.6	0.643967
27.531	NAU3770-187/195	6.43	2.39061	1.11869	1.71499	19.1	0.635962
27.657	MUCS088-170/0	7.44	2.41362	1.05607	1.65803	21.7	0.678779
28.397	NAU1232--205/208	5.4	2.34521	1.15891	1.76751	16.6	0.59315
28.616	CIR196-205/206	7.07	2.42176	1.08058	1.66962	21.2	0.670591
29.315	MUCS088-0/162	6.01	2.37387	1.13427	1.73528	18.1	0.6198
29.846	MGHES-16-0/221	5.77	2.39586	1.17933	1.75233	17.3	0.608261
30.165	NAU2016-0/247	5.84	2.38991	1.16816	1.7482	17.5	0.610872
32.482	NAU3390-213/206	3.27	2.25591	1.31786	1.90224	10.2	0.469024
33.065	BNL1231-208/217	3.29	2.25303	1.30935	1.89897	10.4	0.471838
33.353	NAU2152-221/215	3.08	2.24528	1.33088	1.91335	9.7	0.457198
35.642	Gh288-173/189	3.35	2.21013	1.26383	1.89497	10.6	0.47315
36.435	MUSB0404-320/328	3.04	2.18736	1.28195	1.91384	9.7	0.452703

Acala Maxxa BAC Library

- Developed at Clemson University Genomics Institute using *HindIII* enzyme and ~8X genome coverage.
- Contains 129,024 clones, average insert size of 137 kb.
- Partially end-sequenced 49,982 clones and mined for SSRs.
- 1,164 BAC-end SSR (MUSB) markers and ~600 MUSB markers polymorphic between Upland and Pima cottons.
- Approximately 200 MUSB markers mapped on cotton chromosomes.

Maxxa BAC analysis Methods

Total 22 BACs sequenced and assembled (3-5kb subclones; shotgun sequence from for. and rev. sites to 8X; 800 bp ends using Phred-Phrap and Consed)

13 BACs on Chr11 : 1,458,221bp

9 BACs on chr21 : 867,516 bp

BAC annotation and functional profiling of **bioitic/abiotic stress response elements** by **BLASTN** aligning to the non-redundant protein database: *G. hirsutum* unigene set (GenBank release 165)

Within 98,420 **unigene sequences**:

Chr 11: 746 putative unigenes with e-value of ≤ -20

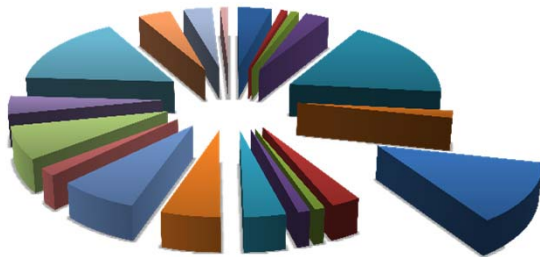
Chr21: 388 putative unigenes with e-value of ≤ -20

Assigned **Gene Ontology** terms

Scanned for conserved protein coding domains using **InterProScan**

Analyzed for molecular interaction and reaction networks with **KEGG biochemical pathway** database

Summary of Biological Processes on Chr11 BACs



- cellular amino acid and derivative metabolic process
- cell cycle
- carbohydrate metabolic process
- cell growth
- **response to stress**
- embryonic development
- translation
- signal transduction
- cell-cell signaling
- generation of precursor metabolites and energy
- cell differentiation
- protein modification process
- transport
- photosynthesis
- cellular component organization
- catabolic process
- DNA metabolic process
- anatomical structure morphogenesis
- lipid metabolic process
- flower development

Chr11 GO stress response

response to external stimulus

response to stress

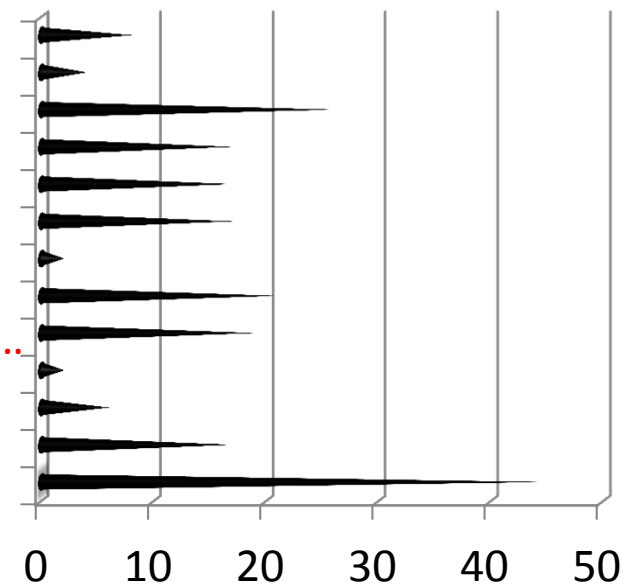
signaling

death

response to endogenous...

cell-cell signaling

response to stimulus



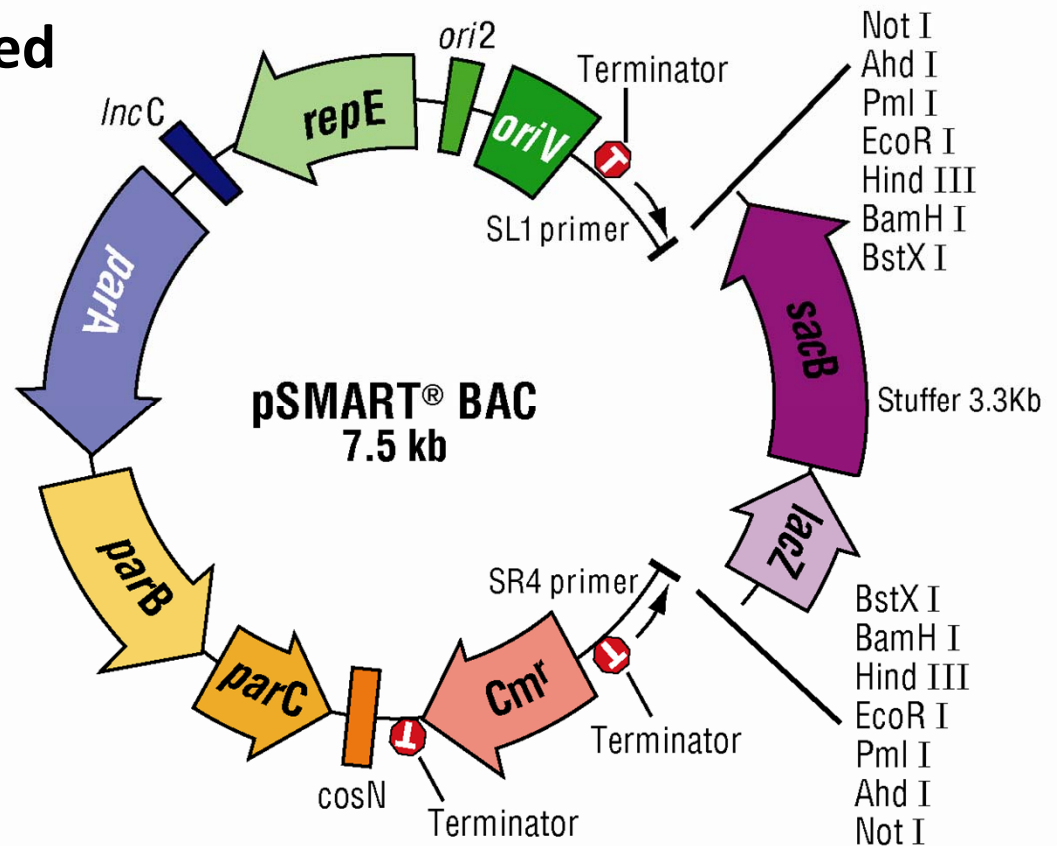
Summary of markers associated with bioprocess on chromosome 21 (239 markers total)

Level	GO ID	Term	# seqs	Graph Scores	Seqs
2	GO:0050896	response to stimulus	11	11.16	NAU2110, HAU3303, CIR275, CIR414, HAU0684, HAU3342, HAU1592, MUSB850, NAU1366, HAU2026, NAU3240
4	GO:0023060	response to external stimulus	4	2.4	HAU2044, BNL137, NAU3748, NAU3895
3	GO:0008219	multicellular organismal process	4	4	HAU1484, HAU3303, NAU1366, DPL0717
3	GO:0009719	response to endogenous stimulus	1	1	NAU2110
3	GO:0009628	response to abiotic stimulus	6	6	HAU2026, HAU0684, HAU3342, HAU1592, NAU1366, NAU3240
2	GO:0016265	multicellular organismal development	4	2.4	HAU1484, HAU3303, NAU1366, DPL0717
3	GO:0023046	response to stress	4	1.44	HAU2044, BNL137, NAU3748, NAU3895
2	GO:0023052	signal transduction	4	0.86	HAU2044, BNL137, NAU3748, NAU3895
2	GO:0032501	cell death	4	3.07	BNL3279, HAU1592, NAU1366, NAU2110
4	GO:0009991	signal transmission	1	1	MUSB850
5	GO:0007165	response to extracellular stimulus	4	4	HAU2044, BNL137, NAU3748, NAU3895
3	GO:0007275	signaling	4	5.12	BNL3279, HAU1592, NAU1366, NAU2110
3	GO:0007154	death	1	1	MUSB850
3	GO:0006950	cell communication	8	8	HAU3303, HAU0684, HAU3342, HAU1592, CIR275, CIR414, MUSB850, NAU1366
3	GO:0009607	signaling process	3	3	HAU3303, CIR275, CIR414
3	GO:0009605	response to external stimulus	1	0.6	MUSB850

NemX BAC library

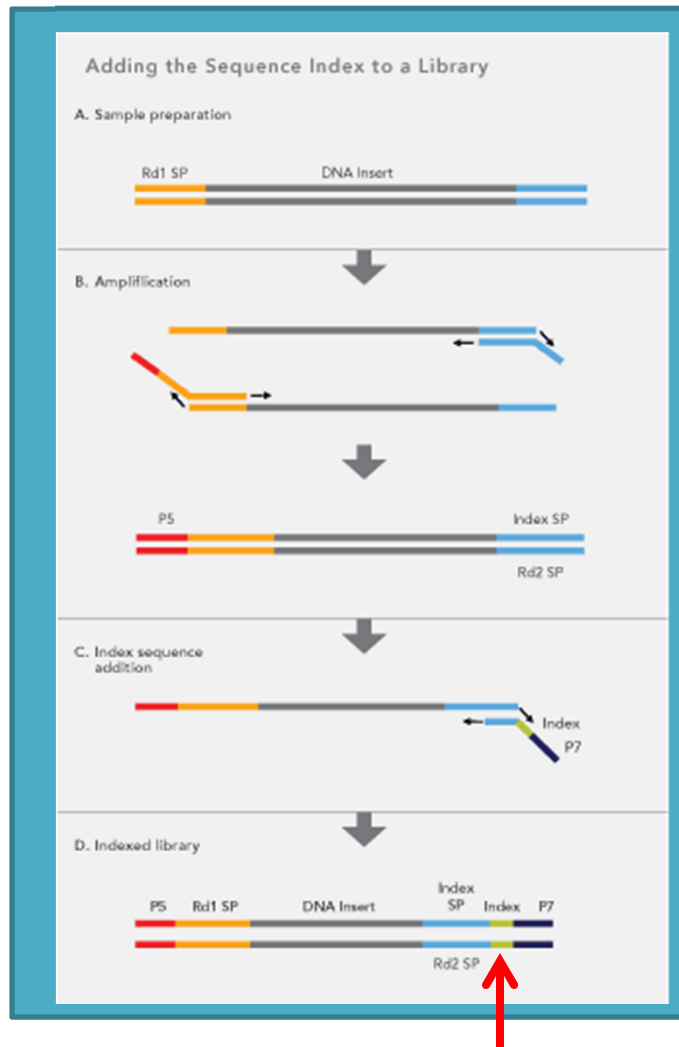
Random Shear BAC library
BAC vector (Lucigen Co.)

- BAC: 158,208 clones arrayed in 412 - 384-well plates
- Ave. insert size: 106 Kb
- 5X genome coverage



Illumina HiSeq2000 platform

Multiplexed Paired-End Sequencing



Bar code
(Index)

NemX BACs

1. One flow cell: 16 adaptors (index)
2. Three flow cells: total **48 clones**.
3. Each flow cell has **9300 Mb** read average
4. Each BAC reading has **> 50x coverage**.
(original BAC is 106 kb ave. size)



Acknowledgements

- Cotton Incorporated (Bob Nichols)
- University of California Discovery Grant Program