

G. longicalyx project introgressing reniform nematode resistance into cotton

Support

Cotton Incorporated

Texas A&M University

USDA-ARS (Cotton Pathology Research Unit)

Scientists and assignments

Hybrid development and breeding.....Al Bell

Cytogenetics & molecular markers.....David Stelly

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Monica Menz

Resistance phenotyping.....Forest Robinson

Objective: Introgress resistance from *G. longicalyx* to produce package of **germplasm + markers** giving industry incentive and means to develop cultivars that **yield** well and **reduce populations** in infested fields

Strategy: Cross *G. hirsutum* with *G. longicalyx* hybrids and **select for resistance** in progeny from repeated backcrosses onto *G. hirsutum* up to BC₆ or BC₇, monitoring progeny cytogenetically and morphologically, then obtain homozygous resistant progeny that **yield** and **lack deleterious recessive exotic genes from hybrids**. Develop markers during introgression beginning with AFLP progressing to SSR or other transportable markers.

Benchmarks and Pitfalls: Introgression into *G. hirsutum* chromosomes, irreversible gene block (e.g. introgression into A genome inversions), low marker density or homology near resistance gene(s), differential expression of multiple resistance genes upon recombination within alien segment.

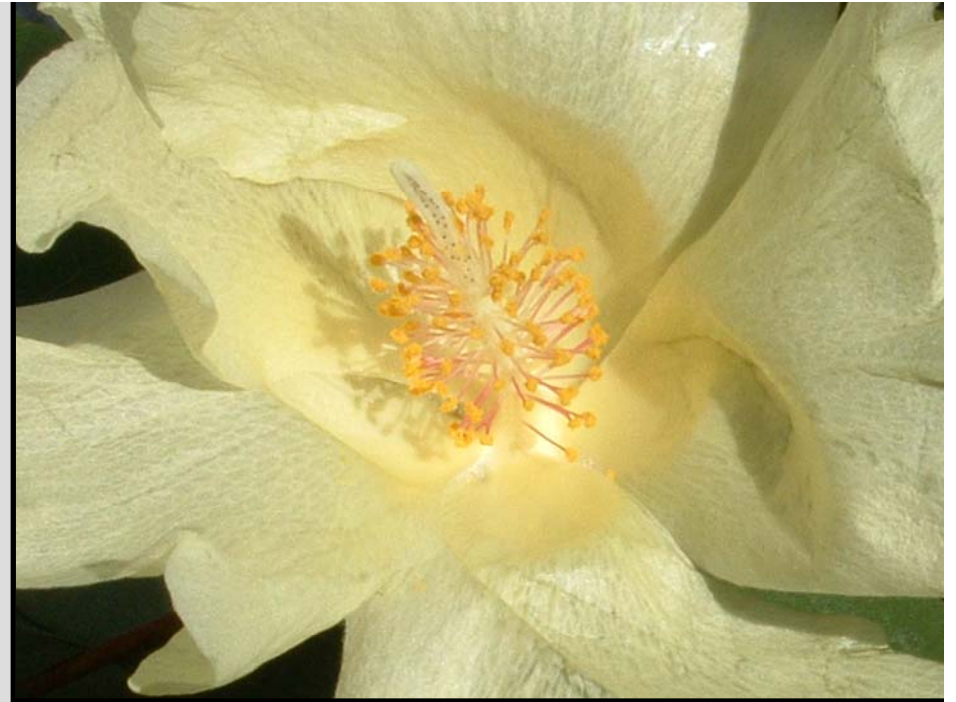
Triple species hybrids (A.A. Bell, 1980's)
used by USDA-ARS to introgress
reniform nematode resistance
from *G. longicalyx* into **upland cotton**

1. **HLA** hybrid (Bell's strategy: substitute **F** for **A**)
(*G. hirsutum* x *G. longicalyx*)² x *G. armourianum*
Genomes: (**AD** x **F**) $\longrightarrow$ (**FAD**)² x **D** $\longrightarrow$ (**FADD**)
2. **HHL** hybrid (Bell's strategy: substitute **F** for **D**)
(*G. hirsutum* x *G. herbaceum*)² x *G. longicalyx*
Genomes: (**AD** x **A**) $\longrightarrow$ (**AAD**)² x **F** $\longrightarrow$ (**AADF**)

[Note: *G. hirsutum* (AADD) 'TM-1' (Deltapine 14) x *G. longicalyx* (FF) hexaploid created by Dr. Meta Brown.]



HHL hybrid – Red petal spots from *G. herbaceum*



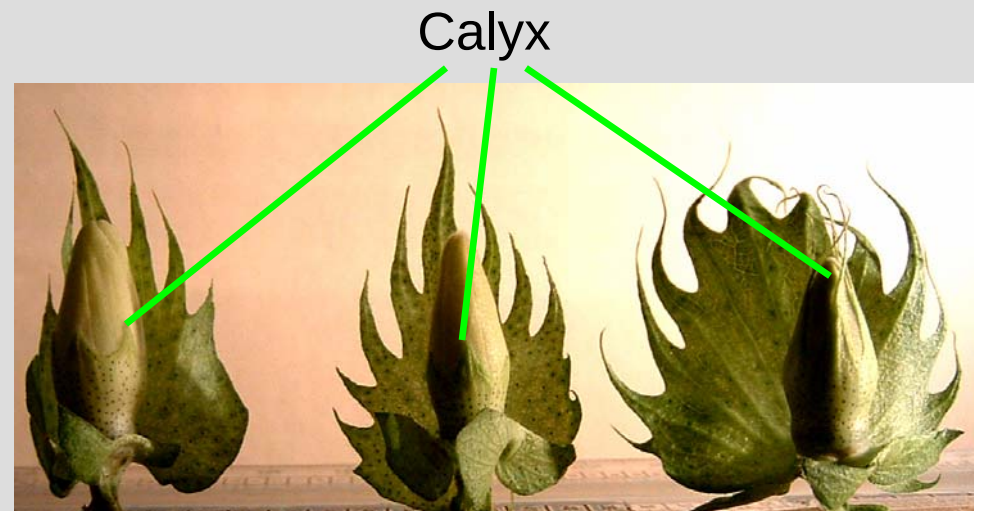
HLA hybrid – Pink staminal filaments from *G. armourianum*



HHL

HLA

Stem pubescence



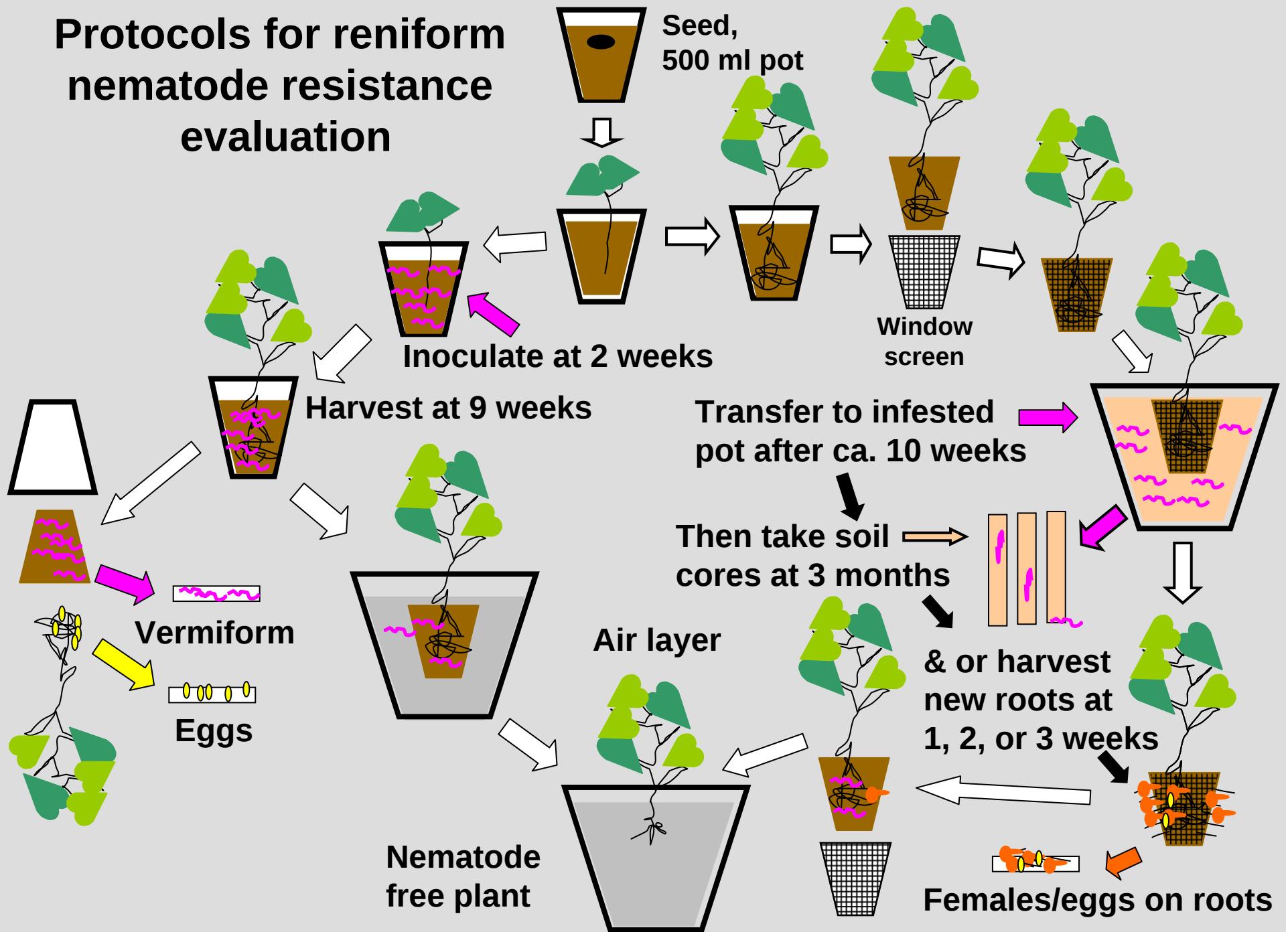
G. hirsutum

BC1 F1



Al Bell with hybrid (Bell on right)

Protocols for reniform nematode resistance evaluation



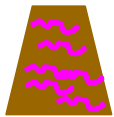
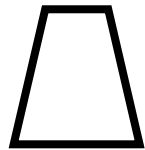
Protocols for reniform nematode resistance evaluation



Inoculate at 2 weeks



Harvest at 9 weeks



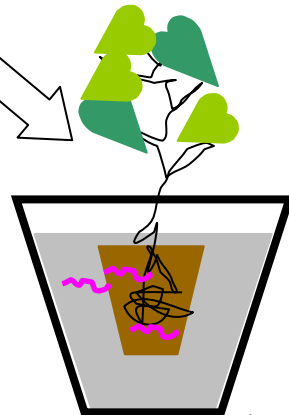
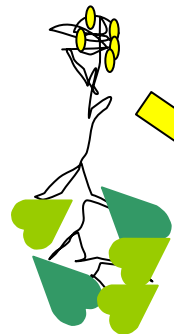
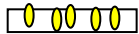
Cores



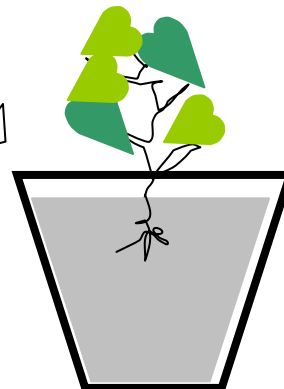
Vermiform



Eggs



Air layer



Nematode
free plant

Progeny status 17 months ago (January 2004)

BC₁ Set A - 1992
(ca. 90 plants, nematode resistance not selected and lost at the BC₂)

**BC₁ F₁
2000-2001**

Of 89 plants from resistant mothers, 41 were immune or highly resistant (28 < 1% ck & 41 < 2% ck)

BC₂ - 2002

Of 58 plants from DP&L 458 mothers, 12 were immune or highly resistant (10 < 1% ck & 12 < 2% ck); pollen from immune or highly resistant BC₁ plants.

BC₁ Set B - 1999
(69 plants, 3% highly fertile, 10 immune, 9 resistant)

**BC₂ Set B
2000-2001**

Of 63 plants from resistant mothers, 5 were immune or highly resistant (3 < 1% ck & 5 < 2% ck); male parents RKN-res.

**BC₃ Set B
2002**

Of 53 plants from D&PL 458 mothers, 19 were immune or highly resistant (12 < 1% ck & 19 < 2% ck); pollen from immune BC₁ plants.

**BC₄ Set B
2003-2004**

Seed in mature bolls of NemX and DP&L 458; pollen from immune BC₃ plants; BC₄'s not yet tested.

BC₁ Set C - 2002
(604 from HLA X NemX, 104 were male fertile; of these 34 (33%) were immune or highly resistant.

BC₂ Set C, 2003

Immune BC₁ used to pollinate NemX and DP&L 458; bolls almost mature)

BC₁ Set D - 2002
(21 from HHL X NemX) 4 were self fertile; of these, 1 (25%) was immune.

BC-2 Set D, 2003

Immune BC₁ used to pollinate NemX and DP&L 458; bolls almost mature)

Total plants phenotyped for resistance = 470, Jan 2004

m		Families in Set B (mixed HLA & HHL)							
		# 2	# 77	# 83	# 84	# 85	# 103	# 122	# 132
	BC2	3	11	7	37	12	29	5	2
	BC3	2	17	7	6	23	3	3	5
	BC4	4	18	6 (10)	32	4 (40)	4 (10)	9	4
	BC5	(10)	4 (20)	(20)	4 (10)	(20)	(10)	(20)	(10)
	BC6		(30)		7 (20)				

Status – June 9, 2005
Number of resistant
plants identified.
(Plants currently being
tested in parentheses)

		Families in Set C (HLA set)													
		# 4	# 18	# 21	# 26	# 34	# 35	# 45	# 61	# 75	# 77	# 81	# 91	# 99	# 103
	BC2	3	4	6	2	2	2	4	3	2	6	5	2	2	3
	BC3	4	4	0	3	3	1	5	4	4	2	5	5	1	4
	BC4														
	BC5														
	BC6														

		Families in Set D (HHL set)					
		# 3	# 5	# 7	# 11	# 14	# 17
	BC2	1	3	3	6	5	5
	BC3	0	4	1	4	2	5
	BC4						
	BC5						

Other generations in Families in Set B								
	# 2	# 77	# 83	# 84	# 85	# 103	# 122	# 132
BC1S1				34		13		
BC2S1						(16)		
BC3S1		19 (108)		57 (204)				
BC4TC		(70)						

Example of resistance assay data set - % of susceptible control

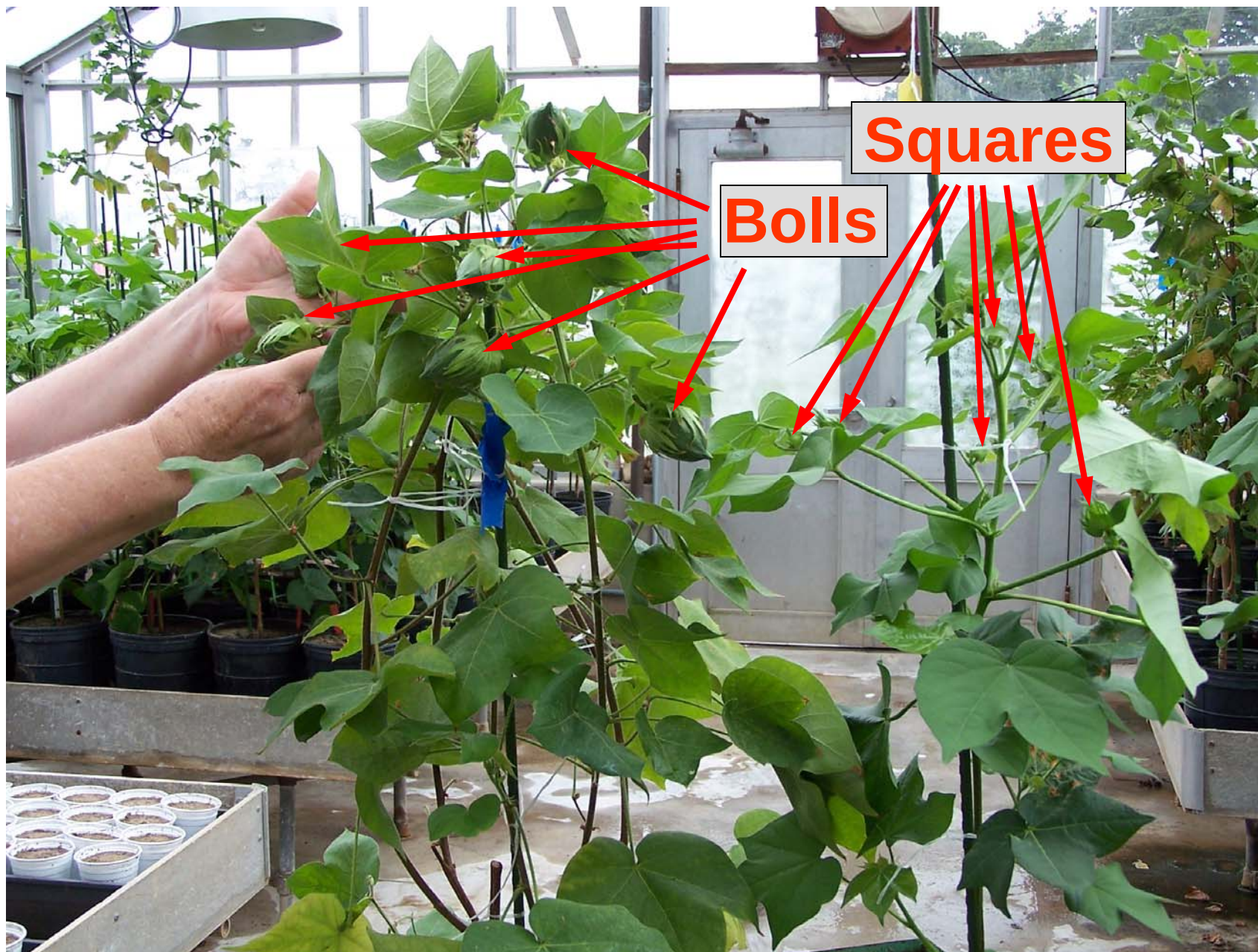
Code	Family	Parent	Generation	Plant number									
				1	2	3	4	5	6	7	8	9	10
95	BC1 77	BC3 1-6 self	BC3S1	4	4	63	51	50	3	3	2	42	93
			BC3S1	37	37	4	4	1	84	60	1	1	45
			BC3S1	6	5	61	54	1	1	109	6	121	3
			BC3S1	3	114	9	2	3	91	66	6	3	96
			BC3S1	120	69	2	46	123					
96	BC1 84	BC3 3-15 self	BC3S1	71	28	1	17	0	0	0	21	0	0
			BC3S1	2	3	38	0	35	1	10	1	0	22
			BC3S1	1	7	2	55	0	0	3	14	1	0
			BC3S1	0	1	0	1	0	0	1	1	0	67
			BC3S1	1	9	0	1	1					
97	BC1 84	BC3 3-24 self	BC3S1	1	1	0	0	94	0	16	5	1	1
			BC3S1	3	2	1	2	2	0	20	1	3	2
			BC3S1	1	1	0	1	0	0	1	1	1	0
98	BC1 2	BC3 1-9 BC*	BC4	4	0	92	69	5	57	87	136	1	78
99	BC1 77	BC4 47-10 BC	BC5	77	115	108	8	21	3	89	123	2	1
100	BC1 77	BC3 4-10 BC	BC4	2	224	9	6	115	78	1	86	99	54
101	BC1 83	BC3 52-7 BC	BC4	4	2	118	1	72	39	0	3	2	134
102	BC1 83	BC2 5-2 BC	BC2	3	77	90	45	149	66	91	2	6	85
103	BC1 85	BC3 11-10 BC	BC4	5	1	1	66	76	73	105	80	29	3
104	BC1 103	BC3 33-3 BC	BC4	128	61	88	3	129	3	54	2	73	4
105	BC1 103	BC2 15-21 BC	BC2	144	73	5	0	88	78	2	4	1	113
106	BC1 122	BC3 19-5 BC	BC4	2	1	37	3	5	3	5	3	3	2
107	BC1 132	BC3 56-8 BC	BC4	127	5	1	38	4	43	79	123	6	0
108	DP-16		Sus. Ck.	157	108	116	116	119	54	105	113	119	98
109	GB-713		Res. Ck.	2	6	3	1	2	14	8	2	5	1

	5% or less
	> 5%, < 1/3
	1/3 to 1/2
	> 50%



Resistant BC₄
plant with bolls

Young resistant BC₆
plant with squares



Resistant BC₄
plant with bolls

Young resistant BC₆
plant with squares