



FOV4 Developments

Christopher Saski
Clemson University
2024 Breeders Tour

Overview of the Systems Genetics Lab

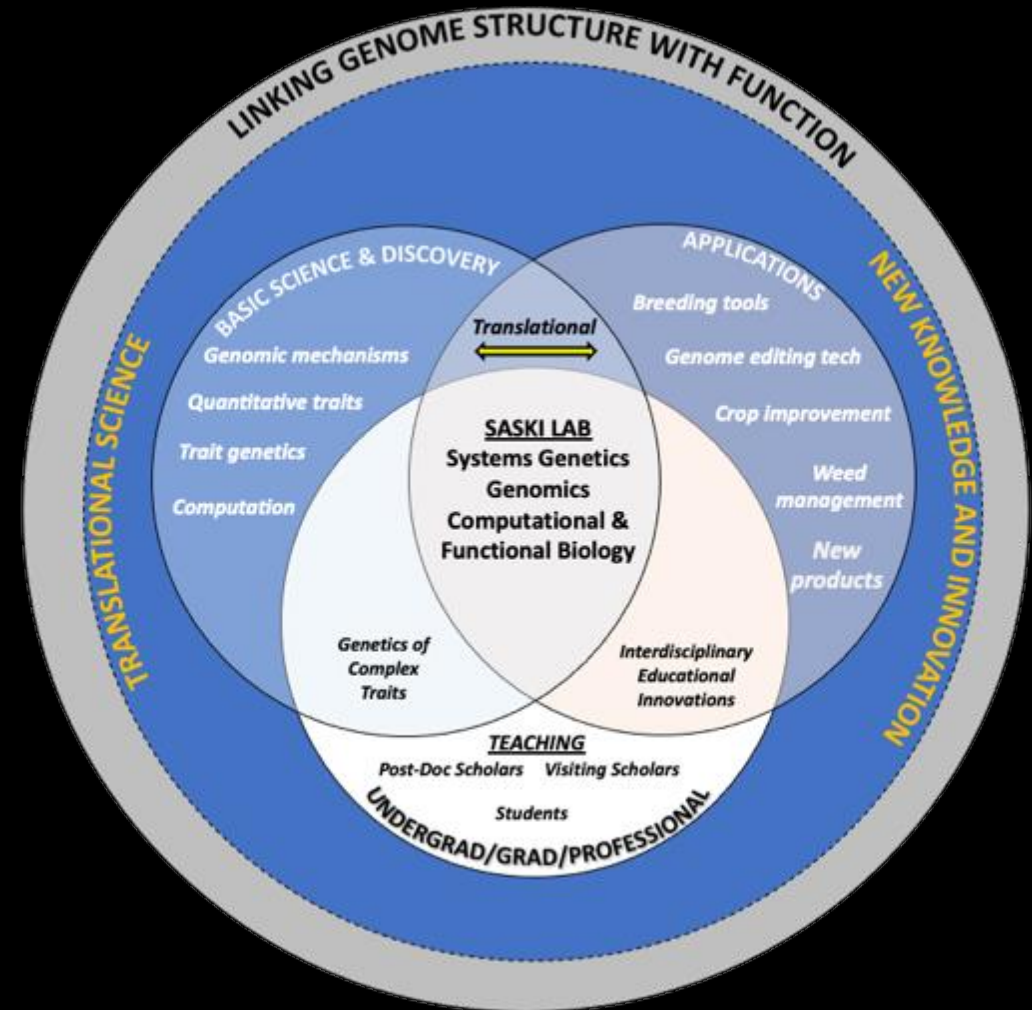
Linking Genome Structure with Function: Resilience, Trait Genetics, Crop Improvement

BASIC RESEARCH – (Disciplinary domains: *Genomics, Genetics, Computational Biology, Genome Engineering, Evolutionary Genomics, Synthetic Biology*)

1. Genetic Architecture and Genomic Mechanisms – Causal alleles underlying simple and quantitative traits
2. Functional Genomics and Genome Engineering – Trait enhancement and engineering

APPLIED RESEARCH – (Plant Breeding, Cropping Systems, Agricultural improvement)

1. Translating Basic Science to Application – DNA Informed breeding, engineered crops, new weed management strategies and agricultural practices



Overview of the Systems Genetics Lab

Linking genome structure with function: Resilience, trait genetics, crop & agricultural improvement

Systems

Fiber crops



Biotic resilience
Developmental genetics
Fiber traits
Advanced breeding

Weeds

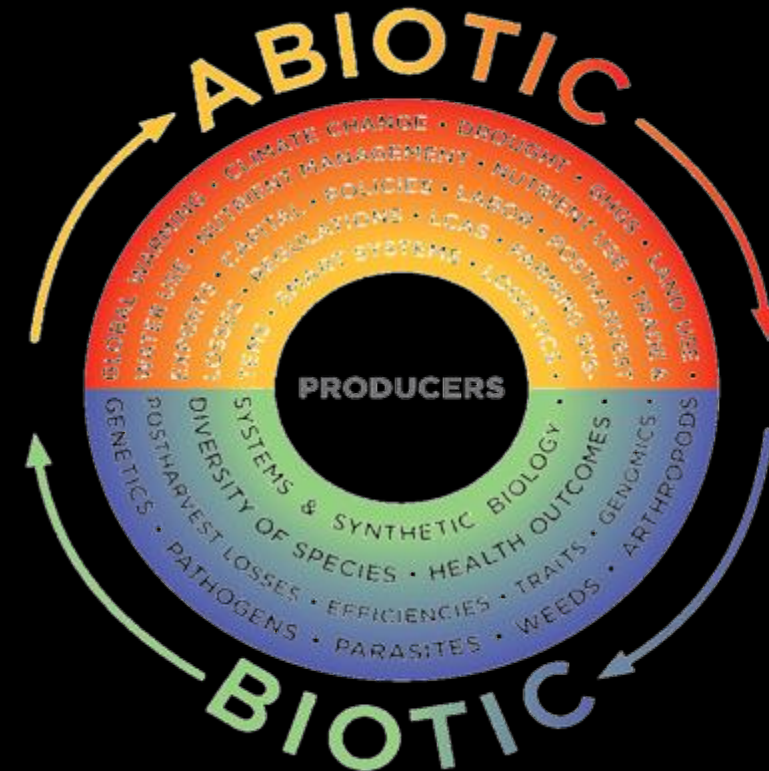


Adaptive traits
Herbicide resistance
Mitigation strategies
Genome dynamics

Rosaceous crops



Biotic resilience
Trait genetics
Rootstock development
DNA-informed breeding





Outline

Primary disease threat to cotton (Fusarium wilt)

Unique FOV4 screening nursery

Genetic mapping for FOV4 resistance in upland

Transcriptomics underlying resistance in pima

Current work & future directions



Fusarium wilt in the US

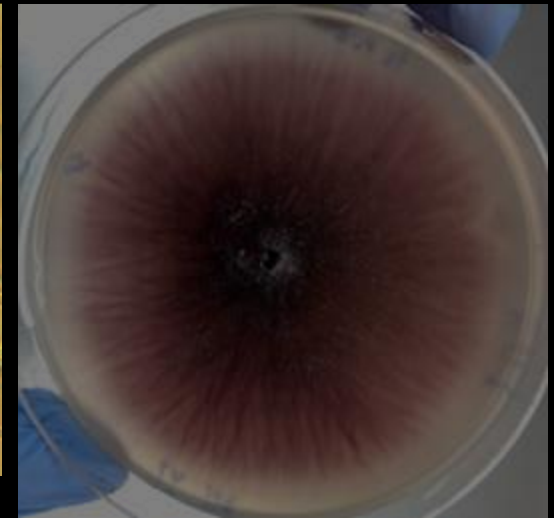
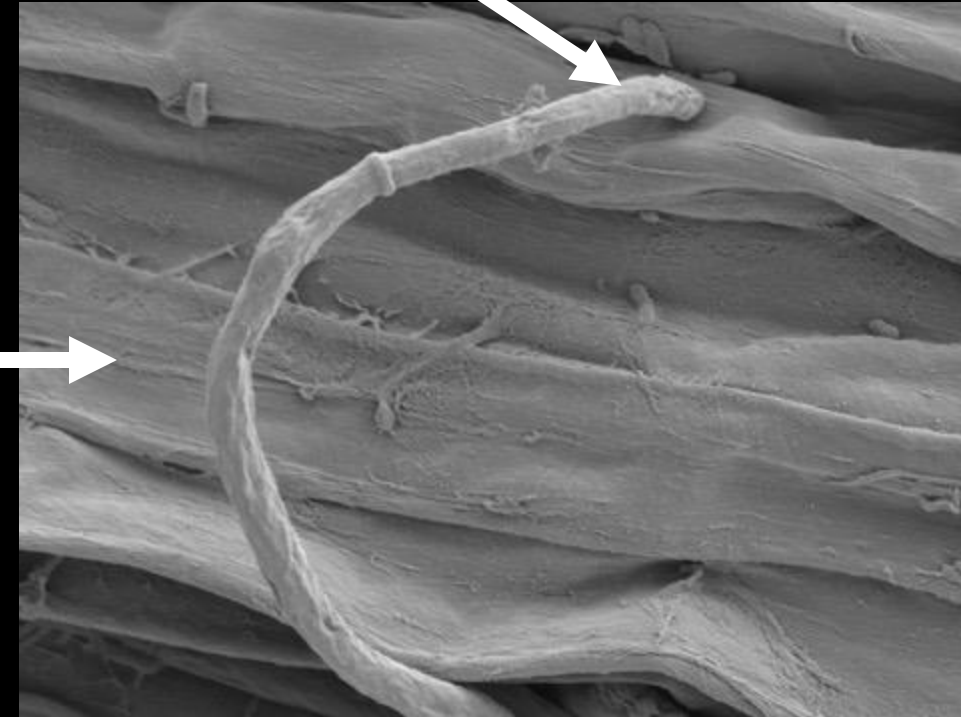
- ✓ *Fusarium oxysporum* f. sp. *vasinfectum*
- ✓ First ID 1892 in Alabama
- ✓ Inoculum dependent
- ✓ No nematodes, no symptoms

Fusarium oxysporum f. sp. *vasinfectum* race 4

- Typical FOV infections of cotton are preceded by a wound
 - Nematode FOV disease complex
 - No nematode, no problems
- First identified in California in 1997 (Olvey)
- FOV4 is nematode independent
- High potential for severe economic damage
- Chlamydospores lie dormant for decades
- Survives as saprophyte

Cotton root
epidermis

FOV4 hyphae



Fusarium wilt symptoms

- Variable based on inoculum density, planting date, pathogen biotype virulence, and age of the host plant
- Seeds germinating in presence of FOV4 spores may die before emergence or immediately after
 - Often confused with damping-off
- Older seedlings display cotyledon drop, leaf chlorosis and necrosis, wilting
- Mature plants are similar and can also show severe stunting and vascular staining



FOV4 consequences

- Field Abandonment
- San Joaquin Valley, California
 - **775,000 down to 40,000** productive acres between 2000 and 2020
- Responsible for 95% stand loss in California from 2007 to 2019
- >50% yield loss in moderately infested fields



Pima breeding efforts

- Early 2000s initial FOV4 breeding focused in pima cotton
 - SUCCESS
- Attempts to introgress this resistance from pima to upland have failed
- Identifying resistant upland germplasm is required



If FOV4 was first identified 20+ years ago, and resistant pima cultivars are developed, why are we still working on upland resistance in 2024?



Upland breeding efforts

- 20 years of research
 - 30+ claims of resistance
 - All fail in high inoculum-load field
- O&A Enterprises
 - FOV4 hot site El Paso, TX
 - Large, diverse germplasm screens
 - Over 10,000 genotypes
- Public releases (O&A Enterprises)
 - U1, U2, U3, and U4

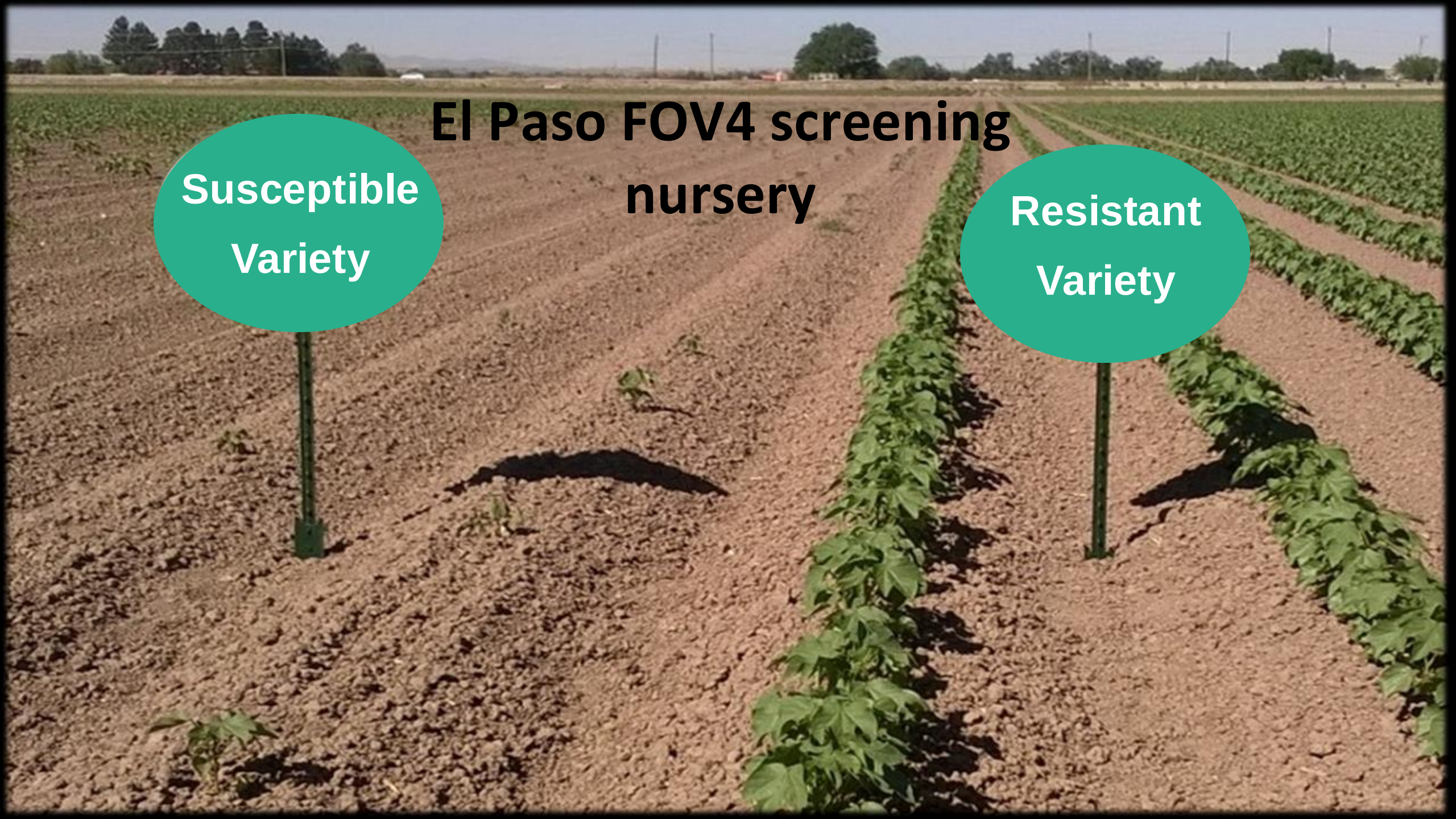




El Paso FOV4 screening nursery

**Susceptible
Variety**

**Resistant
Variety**



Robust & unique field design

	Mod		Tol		Mod		Mod		Tol		Mod		Tol		Mod		56
55	DJ-6-45		DJ-6-94		DJ-6-142		DJ-6-187		Mod		DJ-6-284		DJ-8-41		DJ-8-89		55
54	DJ-6-44		DJ-6-93		DJ-6-141		DJ-6-186		DJ-6-236		DJ-6-283		DJ-8-40		Tol		54
53	DJ-6-43		DJ-6-92		DJ-6-140		DJ-6-185		DJ-6-235		DJ-6-282		Mod		DJ-8-88		53
52	Mod		DJ-6-91		DJ-6-139		DJ-6-184		DJ-6-234		DJ-6-281		DJ-8-39		DJ-8-87		52
51	DJ-6-42		DJ-6-90		DJ-6-138		DJ-6-183		DJ-6-233		Tol		DJ-8-38		DJ-8-86		51
50	DJ-6-41		DJ-6-89		Mod		DJ-6-182		DJ-6-232		DJ-6-280		DJ-8-37		DJ-8-85		50
49	DJ-6-40		DJ-6-88		DJ-6-137		DJ-6-181		DJ-6-231		DJ-6-279		DJ-8-36		DJ-8-84		49
48	DJ-6-39		DJ-6-87		DJ-6-136		DJ-6-180		DJ-6-230		DJ-6-278		DJ-8-35		DJ-8-83		48
47	DJ-6-38		Mod		DJ-6-135		DJ-6-179		DJ-6-229		DJ-6-277		DJ-8-34		DJ-8-82		47
46	DJ-6-37		DJ-6-86		DJ-6-134		DJ-6-178		Tol		DJ-6-276		DJ-8-33		DJ-8-81		46
45	DJ-6-36		DJ-6-85		DJ-6-133		Tol		DJ-6-228		DJ-6-275		DJ-8-32		DJ-8-80		45
44	DJ-6-35		DJ-6-84		DJ-6-132		DJ-6-177		DJ-6-227		DJ-6-274		Tol		DJ-8-79		44
43	DJ-6-34		DJ-6-83		DJ-6-131		DJ-6-176		DJ-6-226		DJ-6-273		DJ-8-31		DJ-8-78		43
42	DJ-6-33		DJ-6-82		DJ-6-130		DJ-6-175		DJ-6-225		DJ-6-272		DJ-8-30		Mod		42
41	Tol		DJ-6-81		DJ-6-129		DJ-6-174		DJ-6-224		DJ-6-271		DJ-8-29		DJ-8-77		41
40	DJ-6-32		DJ-6-80		DJ-6-128		CSX8308		DJ-6-223		Mod		DJ-8-28		DJ-8-76		40
39	DJ-6-31		DJ-6-79		Tol		GA 2016016		DJ-6-222		DJ-6-270		DJ-8-27		DJ-8-75		39
38	DJ-6-30		DJ-6-78		DJ-6-127		SA-3208		DJ-6-221		DJ-6-269		DJ-8-26		DJ-8-74		38
37	DJ-6-29		Tol		DJ-6-126		DJ-6-173		DJ-6-220		DJ-6-268		DJ-8-25		DJ-8-73		37
36	DJ-6-28		DJ-6-77		DJ-6-125		DJ-6-172		DJ-6-219		DJ-6-267		Mod		DJ-8-72		36
35	DJ-6-27	S	DJ-6-76	S	DJ-6-124	S	Mod	S S	DJ-6-218	S	DJ-6-266	S	DJ-8-24	S	DJ-8-71	S	35
34	DJ-6-26	U	DJ-6-75	U	DJ-6-123	U	DJ-6-171	U U	DJ-6-217	U	DJ-6-265	U	DJ-8-23	U	DJ-8-70	U	34
33	DJ-6-25	S	DJ-6-74	S	Mod	S	DJ-6-170	S S	DJ-6-216	S	DJ-6-264	S	DJ-8-22	S	DJ-8-69	S	33
32	DJ-6-24	C	DJ-6-73	C	DJ-6-122	C	DJ-6-169	C C	DJ-6-215	C	DJ-6-263	C	DJ-8-21	C	DJ-8-68	C	32
31	DJ-6-23	E	DJ-6-72	E	DJ-6-121	E	DJ-6-168	E E	DJ-6-214	E	Tol	E	DJ-8-20	E	DJ-8-67	E	31
30	Mod	P	DJ-6-71	P	DJ-6-120	P	DJ-6-167	P P	DJ-6-213	P	DJ-6-262	P	DJ-8-19	P	DJ-8-66	P	30
29	DJ-6-22	T	DJ-6-70	T	DJ-6-119	T	DJ-6-166	T T	DJ-6-212	T	DJ-6-261	T	DJ-8-18	T	Tol		29
28	DJ-6-21	I	DJ-6-69	I	DJ-6-118	I	DJ-6-165	I I	Mod	I	DJ-6-260	I	DJ-8-17	I	DJ-8-65	I	28
27	DJ-6-20	B	DJ-6-68	B	DJ-6-117	B	DJ-6-164	B B	DJ-6-211	B	DJ-6-259	B	DJ-8-16	B	DJ-8-64	B	27
26	DJ-6-19	L	DJ-6-67	L	DJ-6-116	L	Tol	L L	DJ-6-210	L	DJ-6-258	L	DJ-8-15	L	DJ-8-63	L	26
25	DJ-6-18	E	Mod	E	DJ-6-115	E	DJ-6-163	E E	DJ-6-209	E	DJ-6-257	E	DJ-8-14	E	DJ-8-62	E	25
24	DJ-6-17		DJ-6-66		DJ-6-114		DJ-6-162		DJ-6-208		DJ-6-256		DJ-8-13		DJ-8-61		24
23	DJ-6-16		DJ-6-65		DJ-6-113		DJ-6-161		DJ-6-207		DJ-6-255		DJ-8-12		DJ-8-60		23
22	DJ-6-15		DJ-6-64		DJ-6-112		DJ-6-160		DJ-6-206		DJ-6-254		Tol		DJ-8-59		22
21	DJ-6-14		DJ-6-63		DJ-6-111		DJ-6-159		DJ-6-205		DJ-6-253		CSX8308		DJ-8-58		21
20	DJ-6-13		DJ-6-62		Tol		DJ-6-158		DJ-6-204		DJ-6-252		GA 2016016		DJ-8-57		20
19	DJ-6-12		DJ-6-61		DJ-6-110		DJ-6-157		DJ-6-203		DJ-6-251		SA-3208		DJ-8-56		19
18	DJ-6-11		DJ-6-60		DJ-6-109		DJ-6-156		DJ-6-202		Mod		DJ-8-11		DJ-8-55		18
17	Tol		DJ-6-59		DJ-6-108		DJ-6-155		DJ-6-201		DJ-6-250		DJ-8-10		DJ-8-54		17
16	DJ-6-10		DJ-6-58		DJ-6-107		Mod		DJ-6-200		DJ-6-249		DJ-8-9		DJ-8-53		16
15	DJ-6-9		DJ-6-57		DJ-6-106		DJ-6-154		DJ-6-199		DJ-6-248		DJ-8-8		DJ-8-52		15
14	DJ-6-8		DJ-6-56		DJ-6-105		DJ-6-153		Tol		DJ-6-247		DJ-8-7		DJ-8-51		14
13	DJ-6-7		DJ-6-55		DJ-6-104		DJ-6-152		DJ-6-198		DJ-6-246		DJ-8-6		Mod		13
12	DJ-6-6		Tol		DJ-6-103		DJ-6-151		DJ-6-197		DJ-6-245		DJ-8-5		DJ-8-50		12
11	DJ-6-5		DJ-6-54		DJ-6-102		DJ-6-150		DJ-6-196		DJ-6-244		DJ-8-4		DJ-8-49		11
10	DJ-6-4		DJ-6-53		DJ-6-101		DJ-6-149		DJ-6-195		DJ-6-243		Mod		DJ-8-48		10
9	DJ-6-3		DJ-6-52		DJ-6-100		DJ-6-148		DJ-6-194		Tol		DJ-8-3		DJ-8-47		9
8	DJ-6-2		DJ-6-51		Mod		DJ-6-147		DJ-6-193		DJ-6-242		DJ-8-2		DJ-8-46		8
7	DJ-6-1		DJ-6-50		DJ-6-99		DJ-6-146		DJ-6-192		DJ-6-241		DJ-8-1		DJ-8-45		7
6	CSX8308		DJ-6-49		DJ-6-98		DJ-6-145		DJ-6-191		DJ-6-240		DJ-6-288		Tol		6
5	Mod		DJ-6-48		DJ-6-97		DJ-6-144		DJ-6-190		DJ-6-239		DJ-6-287		DJ-8-44		5
4	GA 2016016		DJ-6-47		DJ-6-96		Tol		DJ-6-189		DJ-6-238		DJ-6-286		DJ-8-43		4
3	SA-3208		DJ-6-46		DJ-6-95		DJ-6-143		DJ-6-188		DJ-6-237		DJ-6-285		DJ-8-42		3
2	Mod		Tol		Mod		Tol		Mod		Tol		Mod		Tol		2
1	Tol		Mod		Tol		Mod		Tol		Mod		Tol		Mod		1
	Row 37		Row 38		Row 39		Row 40		Row 41		Row 42		Row 43		Row 44		

6	3	4	4	2	2		6	5	6		4	6	3	5	4
			2				3					3	3	5	
				5				5				2	5		
3					3				5		3	2	3		
	4					5				5					
												1	1		
3						5			4		3	3	1		
												2	3		
											5	5	6		
				5											
			3					5				4	5		
											4	2	5		
									5						
							3					4	5	5	
			5								5	4	3		
	3					3				5					
		2				3					4	4	4		
								6			5	3	3		
3					5				3						
			5								3	5			
											3	2	4		
	3					6			3		5	2			
							5				3	5	2		
											3	3			
			5								5	4	5		
								2							
											3	3			
	1					3					3	3	3	4	
					5										
									3			4	3	4	
											2	2	4		
			4					3							
												3	5	3	
	5					5				4	2	3	5		
4					3				4			5	5	4	
											2	6	5		
4	5	5	5	4	5	5	5	6	4	3	5	5	6		
	5	3	6	6	7	6	6	3	5	5	6	6	5		

Rating	Plant Survival
0	0%
1	1-10%
2	11-20%
3	21-30%
4	31-40%
5	41-50%
6	51-60%
7	61-70%
8	71-80%
9	81-90%
10	91-100%

Geostatistical techniques to account for the heterogeneity of Fusarium wilt inoculum distribution in upland cotton field screening studies



Incorporating geostatistical techniques to improve phenotyping

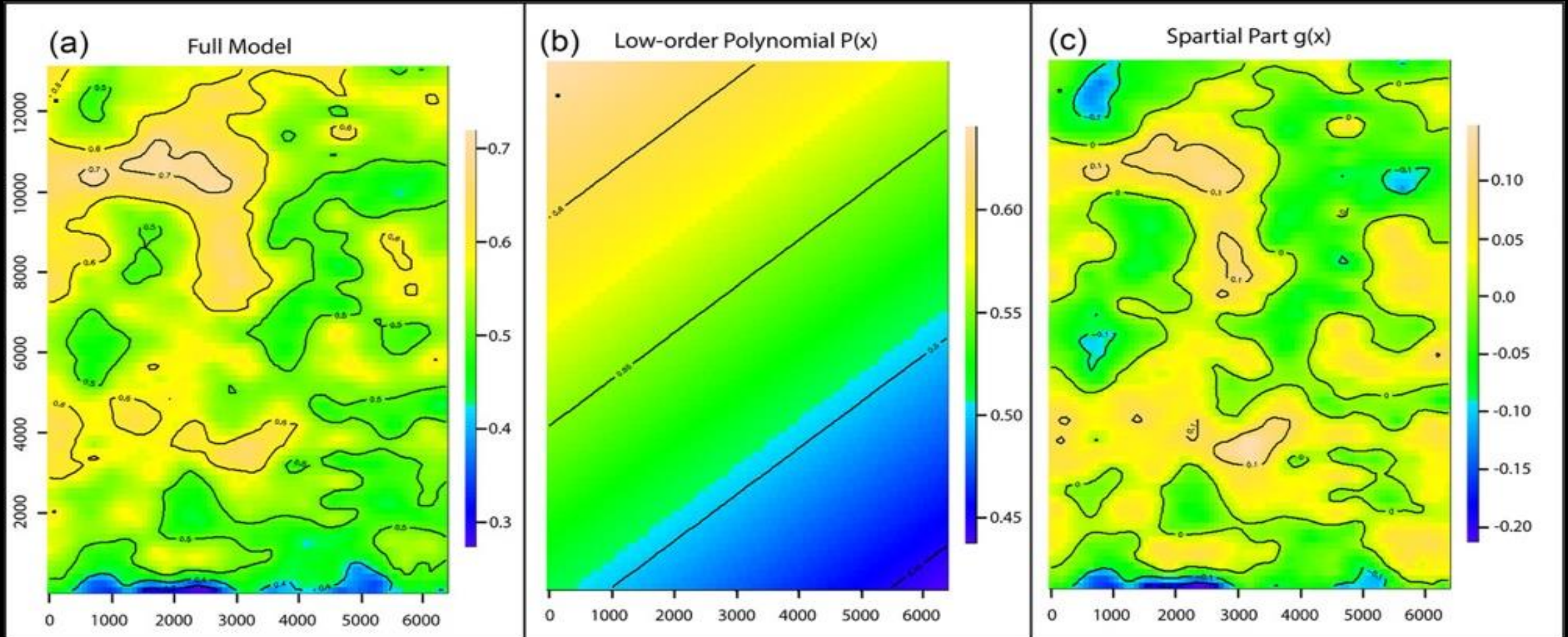
Robust & unique field design

	Mod		Tol		Mod		Mod		Tol		Mod		Tol		Mod		56
55	DJ-6-45		DJ-6-94		DJ-6-142		DJ-6-187		Mod		DJ-6-284		DJ-8-41		DJ-8-89		55
54	DJ-6-44		DJ-6-93		DJ-6-141		DJ-6-186		DJ-6-236		DJ-6-283		DJ-8-40		Tol		54
53	DJ-6-43		DJ-6-92		DJ-6-140		DJ-6-185		DJ-6-235		DJ-6-282		Mod		DJ-8-88		53
52	Mod		DJ-6-91		DJ-6-139		DJ-6-184		DJ-6-234		DJ-6-281		DJ-8-39		DJ-8-87		52
51	DJ-6-42		DJ-6-90		DJ-6-138		DJ-6-183		DJ-6-233		Tol		DJ-8-38		DJ-8-86		51
50	DJ-6-41		DJ-6-89		Mod		DJ-6-182		DJ-6-232		DJ-6-280		DJ-8-37		DJ-8-85		50
49	DJ-6-40		DJ-6-88		DJ-6-137		DJ-6-181		DJ-6-231		DJ-6-279		DJ-8-36		DJ-8-84		49
48	DJ-6-39		DJ-6-87		DJ-6-136		DJ-6-180		DJ-6-230		DJ-6-278		DJ-8-35		DJ-8-83		48
47	DJ-6-38		Mod		DJ-6-135		DJ-6-179		DJ-6-229		DJ-6-277		DJ-8-34		DJ-8-82		47
46	DJ-6-37		DJ-6-86		DJ-6-134		DJ-6-178		Tol		DJ-6-276		DJ-8-33		DJ-8-81		46
45	DJ-6-36		DJ-6-85		DJ-6-133		Tol		DJ-6-228		DJ-6-275		DJ-8-32		DJ-8-80		45
44	DJ-6-35		DJ-6-84		DJ-6-132		DJ-6-177		DJ-6-227		DJ-6-274		Tol		DJ-8-79		44
43	DJ-6-34		DJ-6-83		DJ-6-131		DJ-6-176		DJ-6-226		DJ-6-273		DJ-8-31		DJ-8-78		43
42	DJ-6-33		DJ-6-82		DJ-6-130		DJ-6-175		DJ-6-225		DJ-6-272		DJ-8-30		Mod		42
41	Tol		DJ-6-81		DJ-6-129		DJ-6-174		DJ-6-224		DJ-6-271		DJ-8-29		DJ-8-77		41
40	DJ-6-32		DJ-6-80		DJ-6-128		CSX8308		DJ-6-223		Mod		DJ-8-28		DJ-8-76		40
39	DJ-6-31		DJ-6-79		Tol		GA 2016016		DJ-6-222		DJ-6-270		DJ-8-27		DJ-8-75		39
38	DJ-6-30		DJ-6-78		DJ-6-127		SA-3208		DJ-6-221		DJ-6-269		DJ-8-26		DJ-8-74		38
37	DJ-6-29		Tol		DJ-6-126		DJ-6-173		DJ-6-220		DJ-6-268		DJ-8-25		DJ-8-73		37
36	DJ-6-28		DJ-6-77		DJ-6-125		DJ-6-172		DJ-6-219		DJ-6-267		Mod		DJ-8-72		36
35	DJ-6-27	S	DJ-6-76	S	DJ-6-124	S	Mod	S S	DJ-6-218	S	DJ-6-266	S	DJ-8-24	S	DJ-8-71	S	35
34	DJ-6-26	U	DJ-6-75	U	DJ-6-123	U	DJ-6-171	U U	DJ-6-217	U	DJ-6-265	U	DJ-8-23	U	DJ-8-70	U	34
33	DJ-6-25	S	DJ-6-74	S	Mod	S	DJ-6-170	S S	DJ-6-216	S	DJ-6-264	S	DJ-8-22	S	DJ-8-69	S	33
32	DJ-6-24	C	DJ-6-73	C	DJ-6-122	C	DJ-6-169	C C	DJ-6-215	C	DJ-6-263	C	DJ-8-21	C	DJ-8-68	C	32
31	DJ-6-23	E	DJ-6-72	E	DJ-6-121	E	DJ-6-168	E E	DJ-6-214	E	Tol	E	DJ-8-20	E	DJ-8-67	E	31
30	Mod	P	DJ-6-71	P	DJ-6-120	P	DJ-6-167	P P	DJ-6-213	P	DJ-6-262	P	DJ-8-19	P	DJ-8-66	P	30
29	DJ-6-22	T	DJ-6-70	T	DJ-6-119	T	DJ-6-166	T T	DJ-6-212	T	DJ-6-261	T	DJ-8-18	T	Tol		29
28	DJ-6-21	I	DJ-6-69	I	DJ-6-118	I	DJ-6-165	I I	Mod	I	DJ-6-260	I	DJ-8-17	I	DJ-8-65	I	28
27	DJ-6-20	B	DJ-6-68	B	DJ-6-117	B	DJ-6-164	B B	DJ-6-211	B	DJ-6-259	B	DJ-8-16	B	DJ-8-64	B	27
26	DJ-6-19	L	DJ-6-67	L	DJ-6-116	L	Tol	L L	DJ-6-210	L	DJ-6-258	L	DJ-8-15	L	DJ-8-63	L	26
25	DJ-6-18	E	Mod	E	DJ-6-115	E	DJ-6-163	E E	DJ-6-209	E	DJ-6-257	E	DJ-8-14	E	DJ-8-62	E	25
24	DJ-6-17		DJ-6-66		DJ-6-114		DJ-6-162		DJ-6-208		DJ-6-256		DJ-8-13		DJ-8-61		24
23	DJ-6-16		DJ-6-65		DJ-6-113		DJ-6-161		DJ-6-207		DJ-6-255		DJ-8-12		DJ-8-60		23
22	DJ-6-15		DJ-6-64		DJ-6-112		DJ-6-160		DJ-6-206		DJ-6-254		Tol		DJ-8-59		22
21	DJ-6-14		DJ-6-63		DJ-6-111		DJ-6-159		DJ-6-205		DJ-6-253		CSX8308		DJ-8-58		21
20	DJ-6-13		DJ-6-62		Tol		DJ-6-158		DJ-6-204		DJ-6-252		GA 2016016		DJ-8-57		20
19	DJ-6-12		DJ-6-61		DJ-6-110		DJ-6-157		DJ-6-203		DJ-6-251		SA-3208		DJ-8-56		19
18	DJ-6-11		DJ-6-60		DJ-6-109		DJ-6-156		DJ-6-202		Mod		DJ-8-11		DJ-8-55		18
17	Tol		DJ-6-59		DJ-6-108		DJ-6-155		DJ-6-201		DJ-6-250		DJ-8-10		DJ-8-54		17
16	DJ-6-10		DJ-6-58		DJ-6-107		Mod		DJ-6-200		DJ-6-249		DJ-8-9		DJ-8-53		16
15	DJ-6-9		DJ-6-57		DJ-6-106		DJ-6-154		DJ-6-199		DJ-6-248		DJ-8-8		DJ-8-52		15
14	DJ-6-8		DJ-6-56		DJ-6-105		DJ-6-153		Tol		DJ-6-247		DJ-8-7		DJ-8-51		14
13	DJ-6-7		DJ-6-55		DJ-6-104		DJ-6-152		DJ-6-198		DJ-6-246		DJ-8-6		Mod		13
12	DJ-6-6		Tol		DJ-6-103		DJ-6-151		DJ-6-197		DJ-6-245		DJ-8-5		DJ-8-50		12
11	DJ-6-5		DJ-6-54		DJ-6-102		DJ-6-150		DJ-6-196		DJ-6-244		DJ-8-4		DJ-8-49		11
10	DJ-6-4		DJ-6-53		DJ-6-101		DJ-6-149		DJ-6-195		DJ-6-243		Mod		DJ-8-48		10
9	DJ-6-3		DJ-6-52		DJ-6-100		DJ-6-148		DJ-6-194		Tol		DJ-8-3		DJ-8-47		9
8	DJ-6-2		DJ-6-51		Mod		DJ-6-147		DJ-6-193		DJ-6-242		DJ-8-2		DJ-8-46		8
7	DJ-6-1		DJ-6-50		DJ-6-99		DJ-6-146		DJ-6-192		DJ-6-241		DJ-8-1		DJ-8-45		7
6	CSX8308		DJ-6-49		DJ-6-98		DJ-6-145		DJ-6-191		DJ-6-240		DJ-6-288		Tol		6
5	Mod		DJ-6-48		DJ-6-97		DJ-6-144		DJ-6-190		DJ-6-239		DJ-6-287		DJ-8-44		5
4	GA 2016016		DJ-6-47		DJ-6-96		Tol		DJ-6-189		DJ-6-238		DJ-6-286		DJ-8-43		4
3	SA-3208		DJ-6-46		DJ-6-95		DJ-6-143		DJ-6-188		DJ-6-237		DJ-6-285		DJ-8-42		3
2	Mod		Tol		Mod		Tol		Mod		Tol		Mod		Tol		2
1	Tol		Mod		Tol		Mod		Tol		Mod		Tol		Mod		1
	Row 37		Row 38		Row 39		Row 40		Row 41		Row 42		Row 43		Row 44		

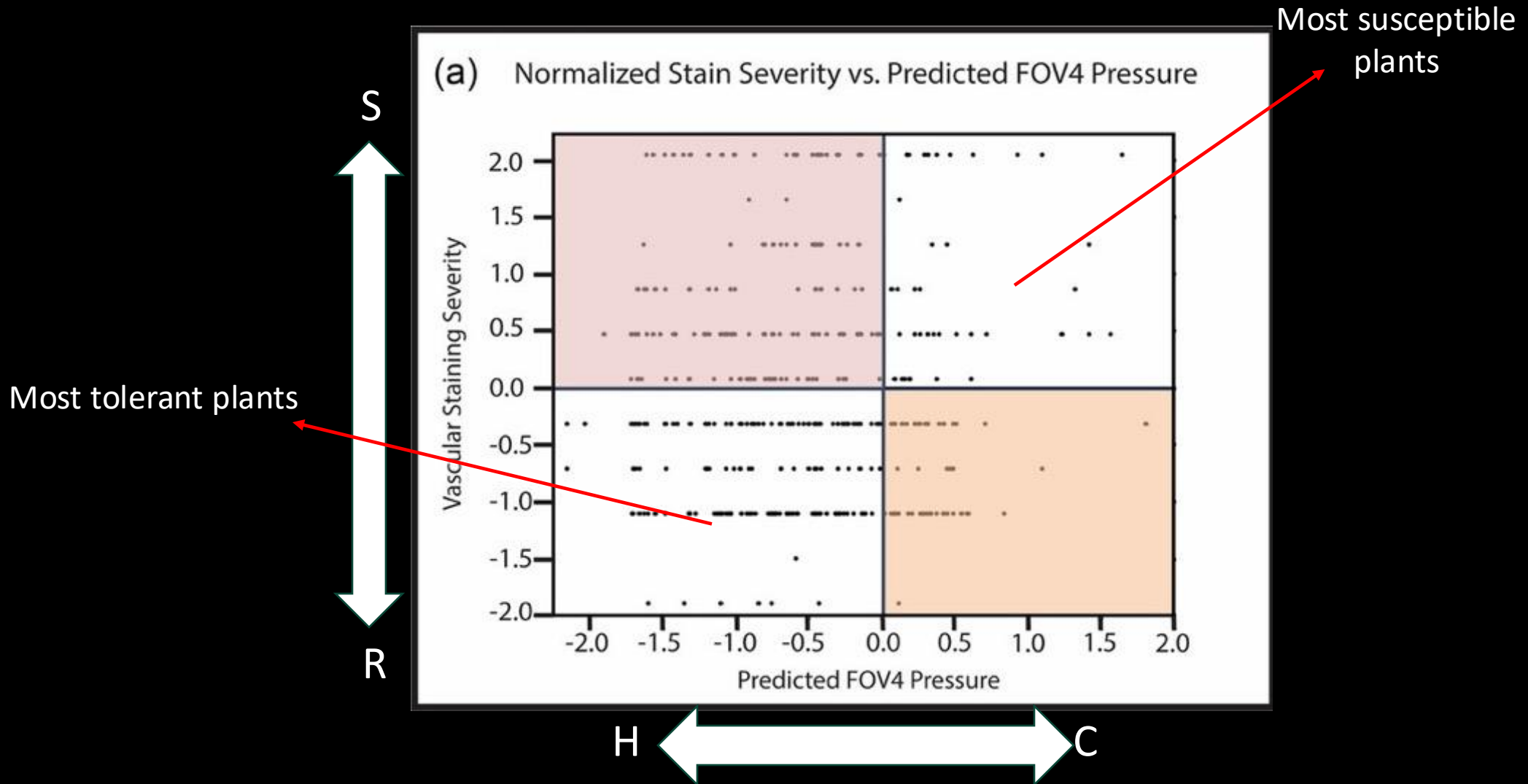
6	3	4	4	2	2		6	5	6		4	6	3	5	4
			2				3					3	3	5	
				5				5				2	5		
3					3				5		3	2	3		
	4					5				5					
												1	1		
3						5			4		3	3	1		
												2	3		
											5	5	6		
				5											
			3					5				4	5		
											4	2	5		
									5						
							3					4	5	5	
			5								5	4	3		
	3					3				5					
		2				3					4	4	4		
								6			5	3	3		
3					5				3						
			5								3	5			
											3	2	4		
	3					6			3		5	2			
							5				3	5	2		
											3	3			
			5								5	4	5		
								2							
											3	3			
	1					3					3	3	3	4	
					5										
									3			4	3	4	
											2	2	4		
			4					3							
												3	5	3	
	5					5				4	2	3	5		
4					3				4			5	5	4	
											2	6	5		
4	5	5	5	4	5	5	5	6	4	3	5	5	6		
	5	3	6	6	7	6	6	3	5	5	6	6	5		

Rating	Plant Survival
0	0%
1	1-10%
2	11-20%
3	21-30%
4	31-40%
5	41-50%
6	51-60%
7	61-70%
8	71-80%
9	81-90%
10	91-100%

Kriging interpolation



Can we use math to sub-select from an early generation population?



Bi-parental cross between 'U1' and 'CSX8308' (DJ8) :



'CSX8308'



'U1'

288+ F3 individuals

Selection of 94 individual
DJ8 plants by inoculum
adjusted phenotype

1/3
Resistant

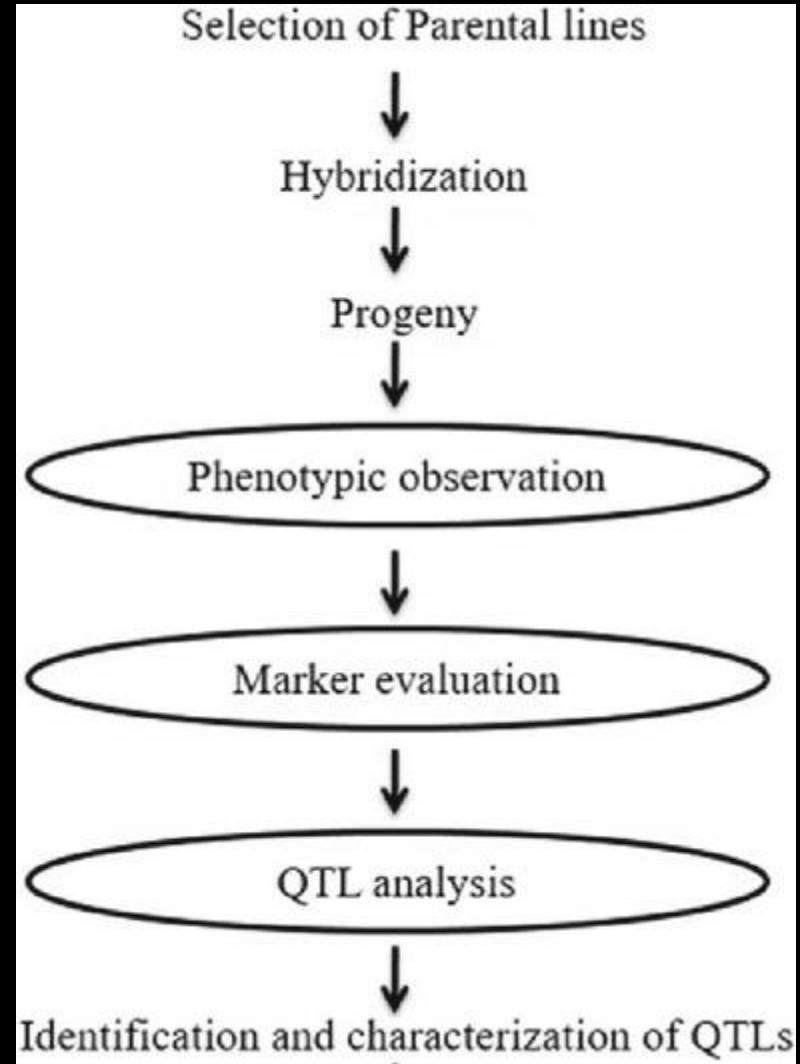
1/3
Moderate

1/3
Susceptible

I
N
F
O
R
M
E
D

S
E
L
E
C
T
I
O
N

FOV4 QTL discovery



Reference grade whole genome sequences

Adjusted with inoculum distribution info

Whole genome sequencing of F3s

Gene discovery

Unique genomic resources

FOV4 Susceptible

A CSX8308

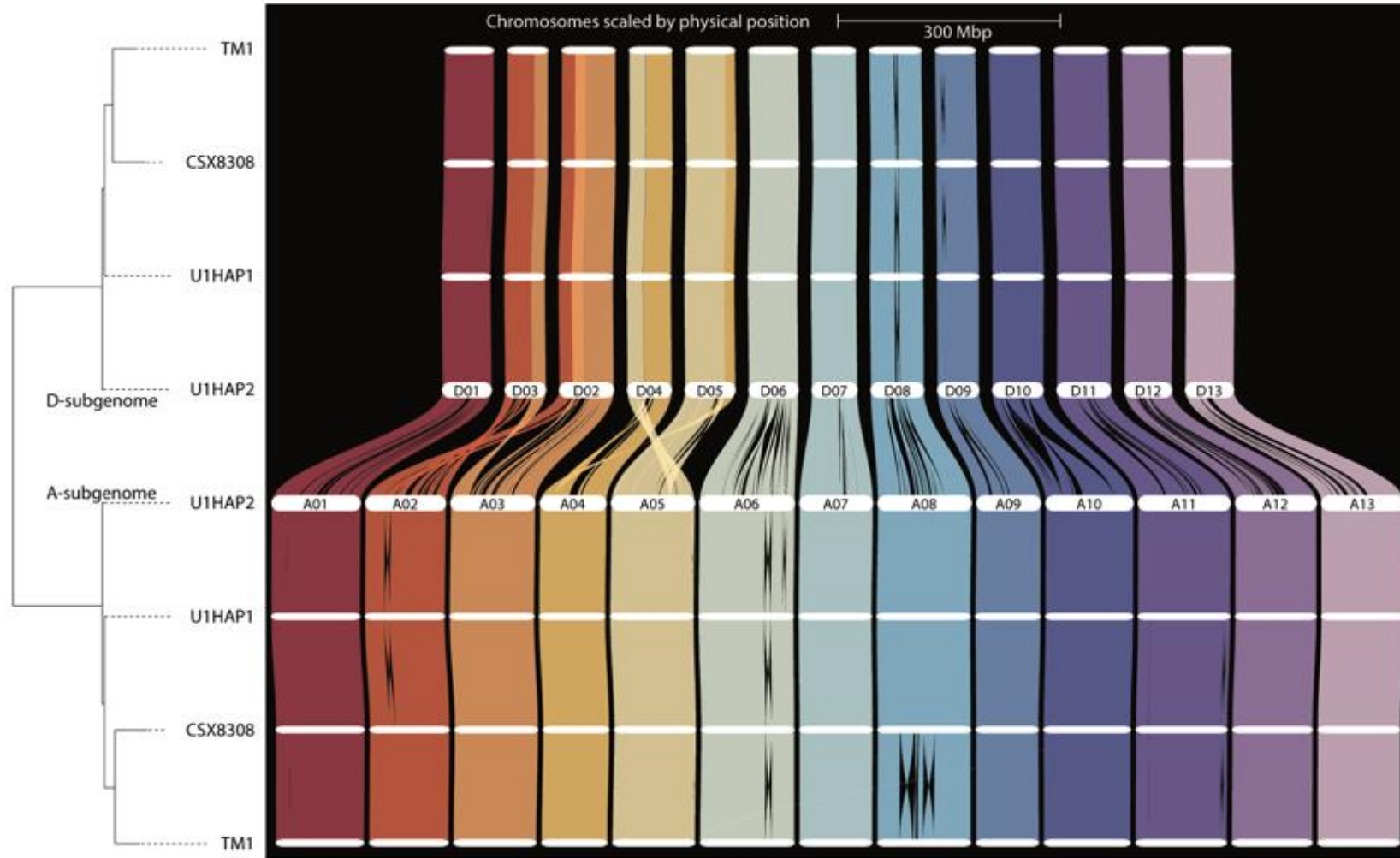


B U1



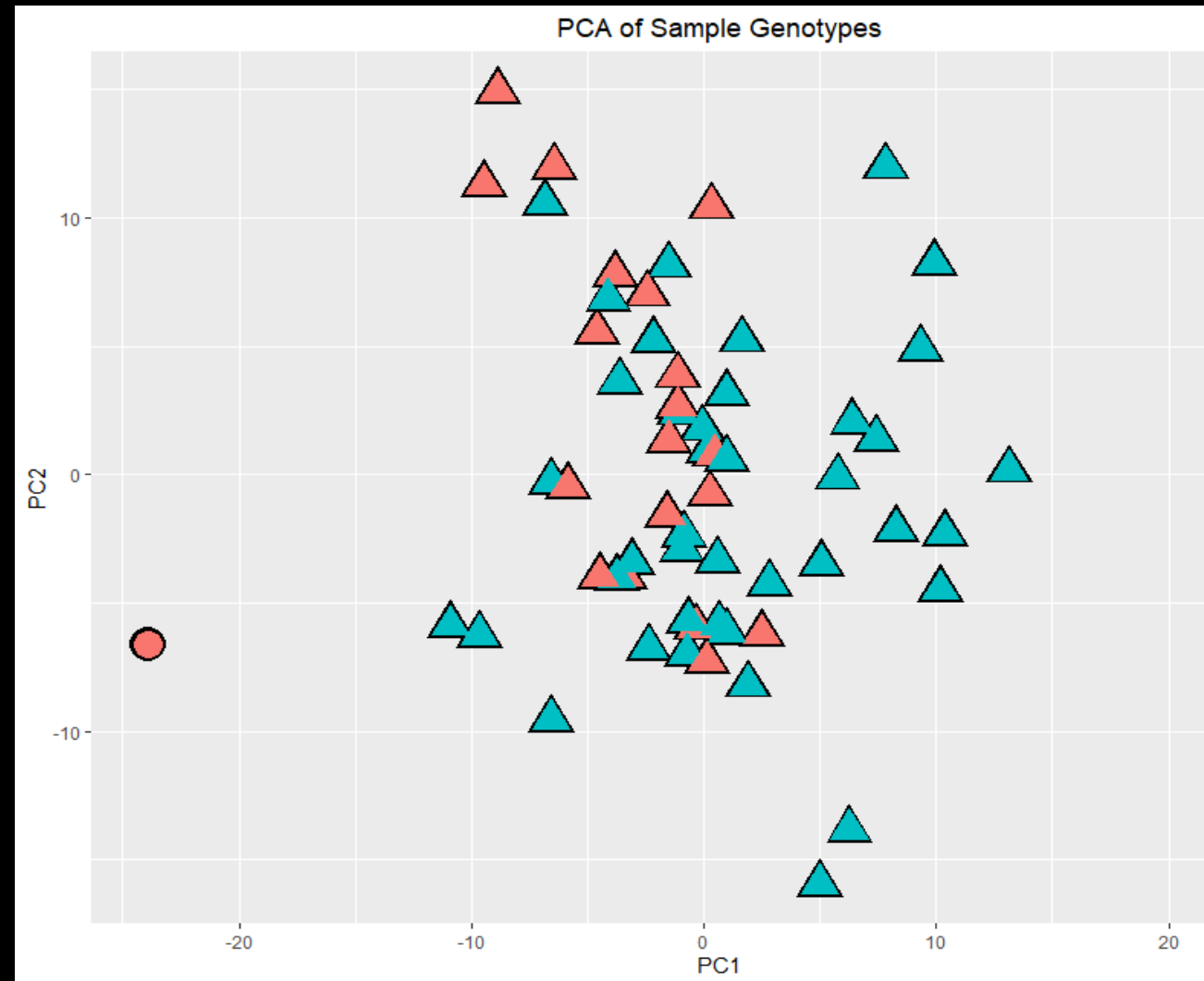
FOV4 Resistant

C Synteny map and single-copy gene tree across four cotton genomes, split by subgenome



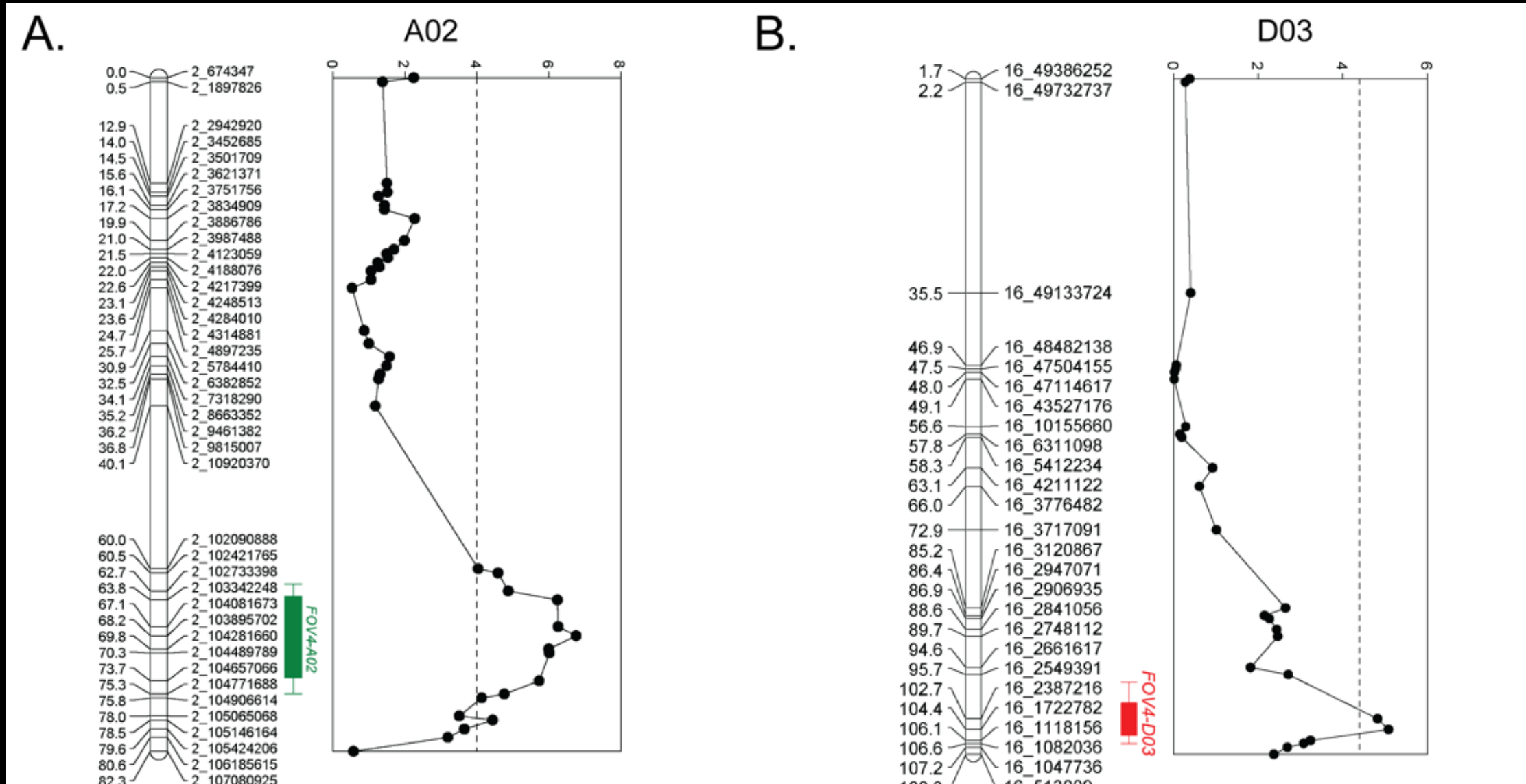
Skim sequencing F3 segregants

- 3-5X sequence coverage of F3
- Selected SNPs between parents that were in homozygous states
- Filtered for read depth, linkage disequilibrium, and minor allele frequency
- Generated 1300 SNP-based markers customized for our population

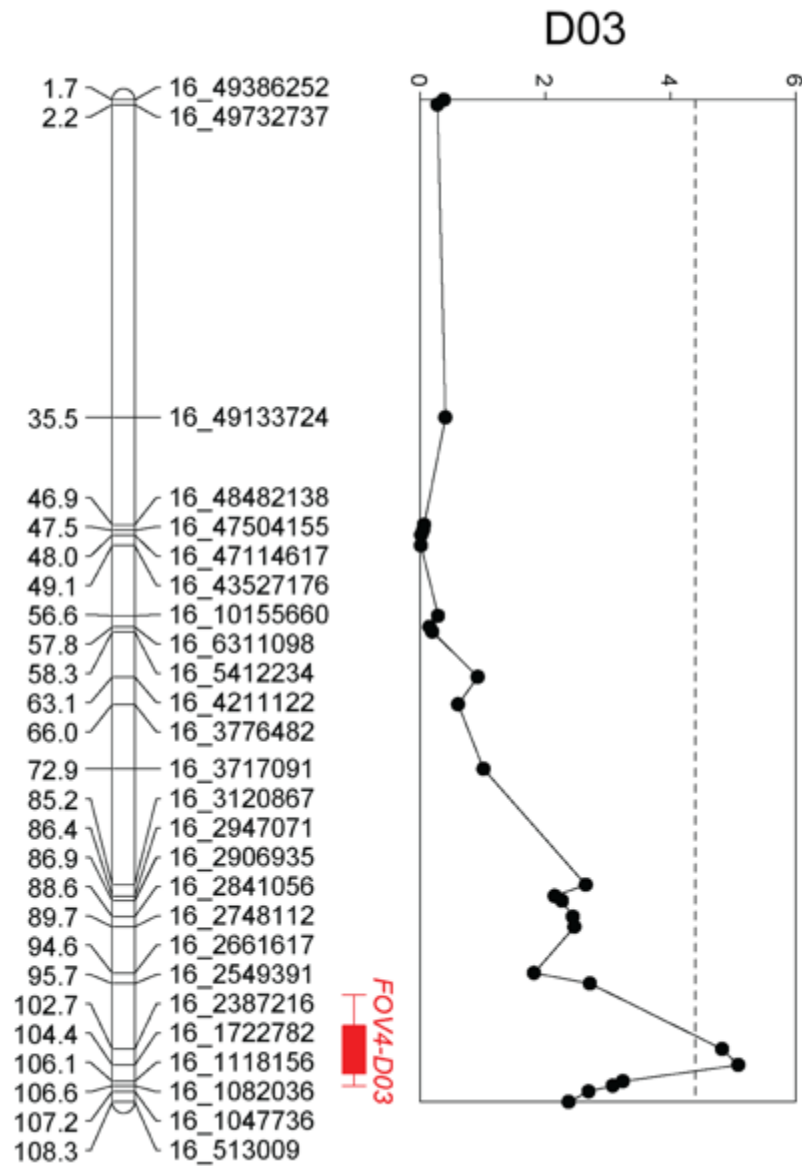


- CSX8308
- U1
- △ F3
- Susceptible
- Resistant

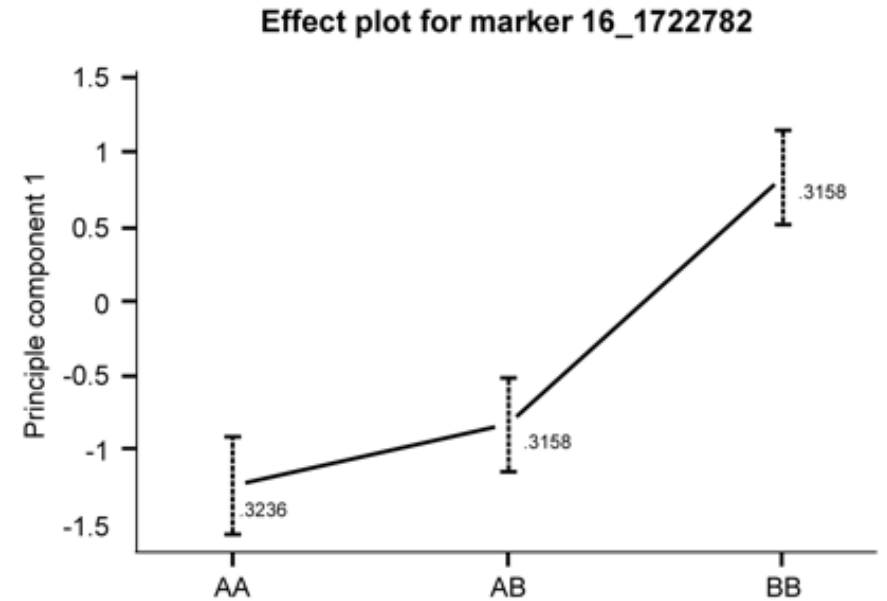
Two major QTLs significantly associated with FOV4 resistance



B.



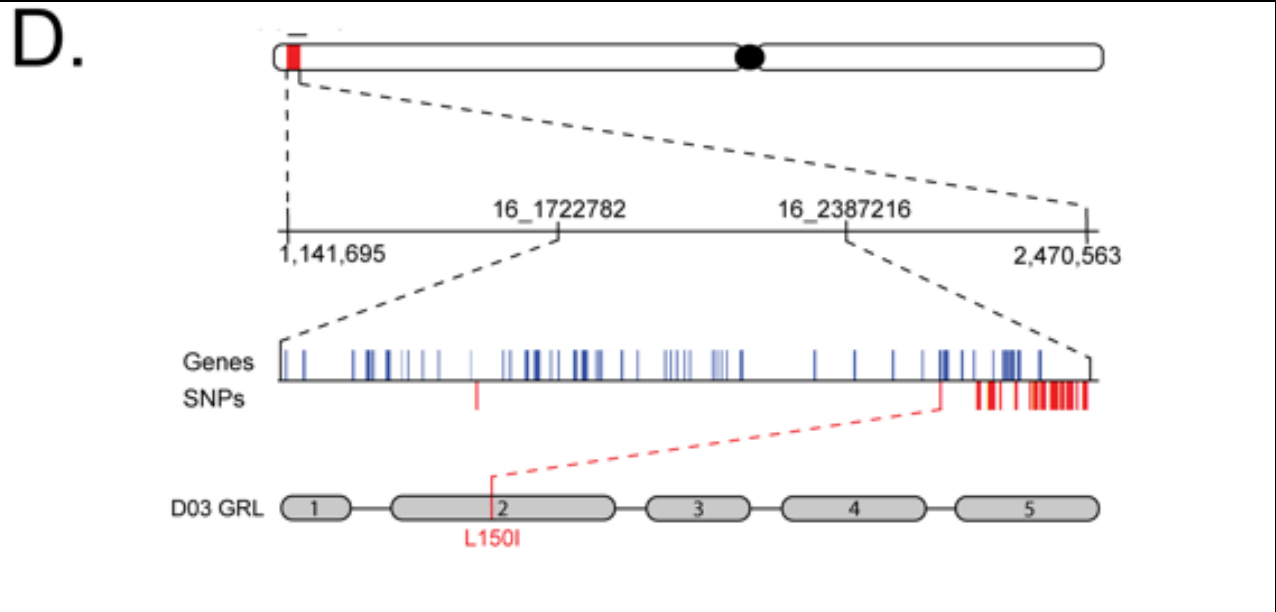
F.



QTL on chromosome D03

1.3 Megabases
102 genes

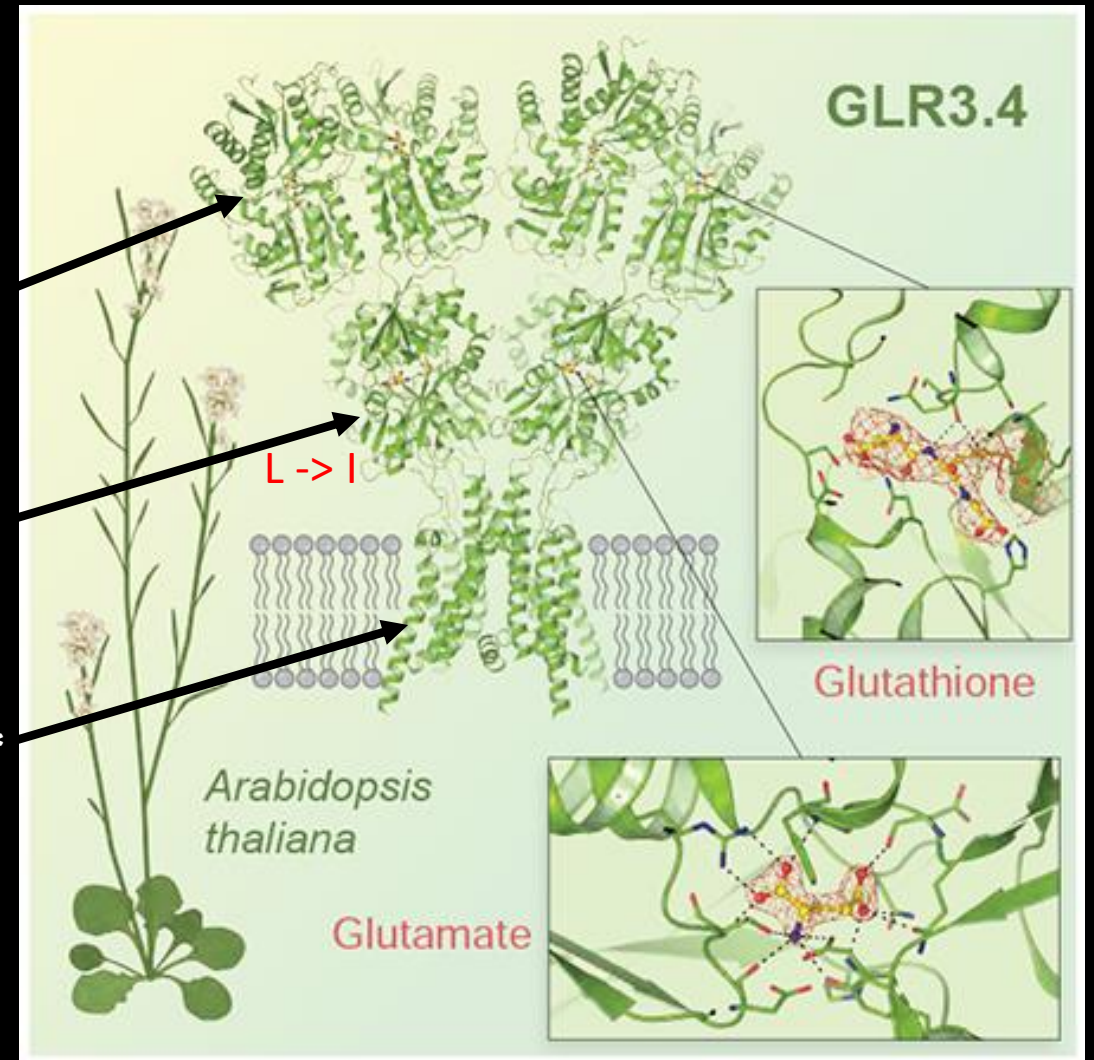
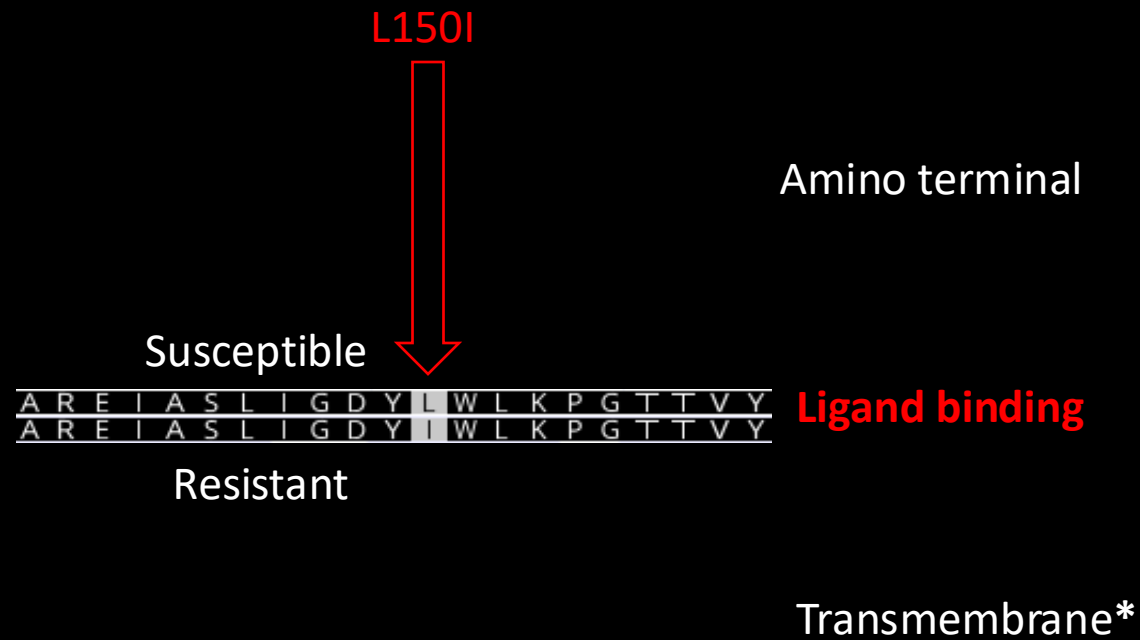
D03 QTL gene discovery



Most Resistant F3s

CHROM	POS	REF	ALT	EFF[*].GENE	Gene IPS	CSX8308	U1	DJ8-22	DJ8-65	DJ8-64	DJ8-31	DJ8-66	DJ8-72	DJ8-80	DJ8-57	DJ8-75
D03	705170	A	G	GhCSX8308.D03G011500	#N/A	BB	AA	AA	AA	AA	./.	BB	AB	BB	AA	AB
D03	806292	T	C	GhCSX8308.D03G012600	Oxidoreductase-like protein, N-terminal	BB	AA	AA	AA	AA	BB	BB	AA	AB	AA	AB
D03	813094	G	C	GhCSX8308.D03G012700	Domain of unknown function (DUF702)	BB	AA	AA	AA	AA	BB	BB	AB	./.	AA	AB
D03	844501	A	G	GhCSX8308.D03G012900	Ribosomal RNA adenine dimethylase	BB	AA	AA	AA	./.	AB	BB	AB	AB	AA	AB
D03	1045210	A	G	GhCSX8308.D03G015700	#N/A	BB	AA	AA	AA	AA	BB	BB	AB	AA	AA	AB
D03	1046449	T	A	GhCSX8308.D03G015700	#N/A	BB	AA	AA	AA	./.	./.	./.	AA	AB	AA	./.
D03	1069455	G	T	GhCSX8308.D03G015900	Clathrin adaptor complex small chain	BB	AA	AA	AA	AA	BB	BB	AB	AB	AA	AB
D03	1071112	A	G	GhCSX8308.D03G016000	Lysine methyltransferase	BB	AA	AA	AA	AA	BB	BB	./.	BB	./.	AB
D03	1074897	C	T	GhCSX8308.D03G016100	CNH domain	BB	AA	AA	AA	./.	BB	BB	./.	BB	AA	AB
D03	1075536	A	T	GhCSX8308.D03G016100	CNH domain	BB	AA	AA	AA	AA	BB	BB	AB	./.	./.	./.
D03	1077111	G	A	GhCSX8308.D03G016100	CNH domain	BB	AA	AA	AA	AA	BB	BB	AB	AB	AA	BB
D03	1101124	C	T	GhCSX8308.D03G016400	GRAS domain family	BB	AA	AA	AA	AA	BB	BB	AB	AB	AA	AB
D03	1722782	Bin Marker	5.084	#N/A	#N/A	BB	AA	AA	AA	AA	AB	AA	AA	AA	AA	AB
D03	2264277	T	G	GhCSX8308.D03G026100	Ligand-gated ion channel	BB	AA	AA	AA	AA	AB	AA	AA	AA	AA	AB
D03	2387216	Bin Marker	4.828375	#N/A	#N/A	BB	AA	AA	AA	AA	AB	AA	AA	AA	AA	AB
D03	2470563	A	T	GhCSX8308.D03G027800	Plant zinc cluster domain	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB
D03	2526112	G	T	GhCSX8308.D03G028200	Cyclin, N-terminal domain	BB	AA	./.	./.	BB	./.	./.	BB	./.	./.	./.
D03	2739208	A	T	GhCSX8308.D03G030300	Protein kinase domain	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB
D03	2896016	G	T	GhCSX8308.D03G031300	GRAS domain family	BB	AA	BB	BB	AA	AB	./.	./.	AB	./.	AB
D03	2902573	C	T	GhCSX8308.D03G031400	Glycosyl hydrolases family 43	BB	AA	AB	BB	AA	AA	BB	AA	./.	./.	AA
D03	2914819	A	G	GhCSX8308.D03G031600	Myb-like DNA-binding domain	BB	AA	AB	BB	AA	AB	BB	AA	AB	BB	AB
D03	2937240	A	G	GhCSX8308.D03G031800	RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain)	BB	AA	BB	BB	AA	AA	./.	./.	./.	./.	./.

Plant glutamate receptors



Adv Sci (Weinh). 2021 Apr; 8(7): 2002723.

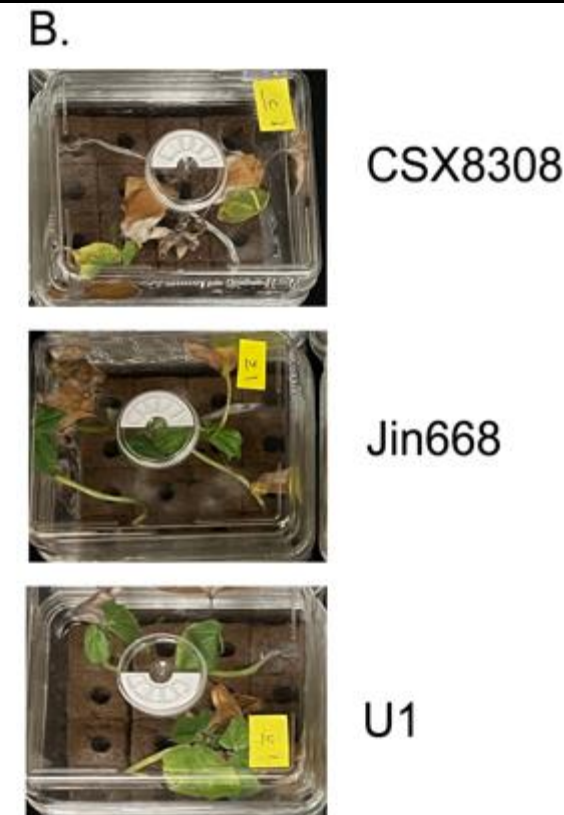
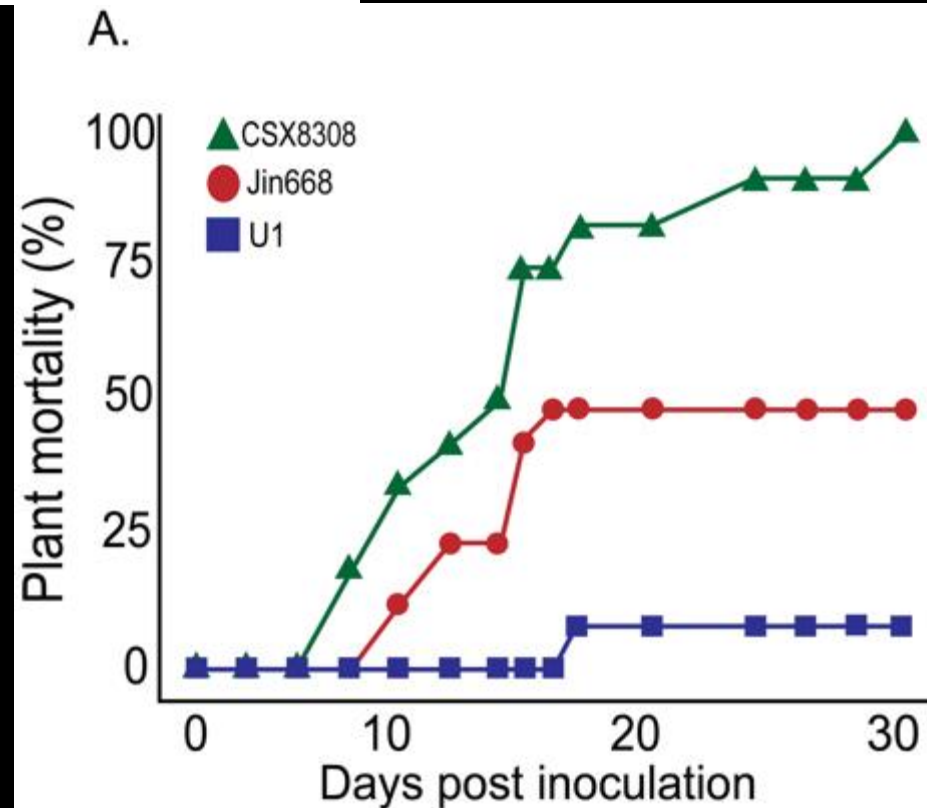
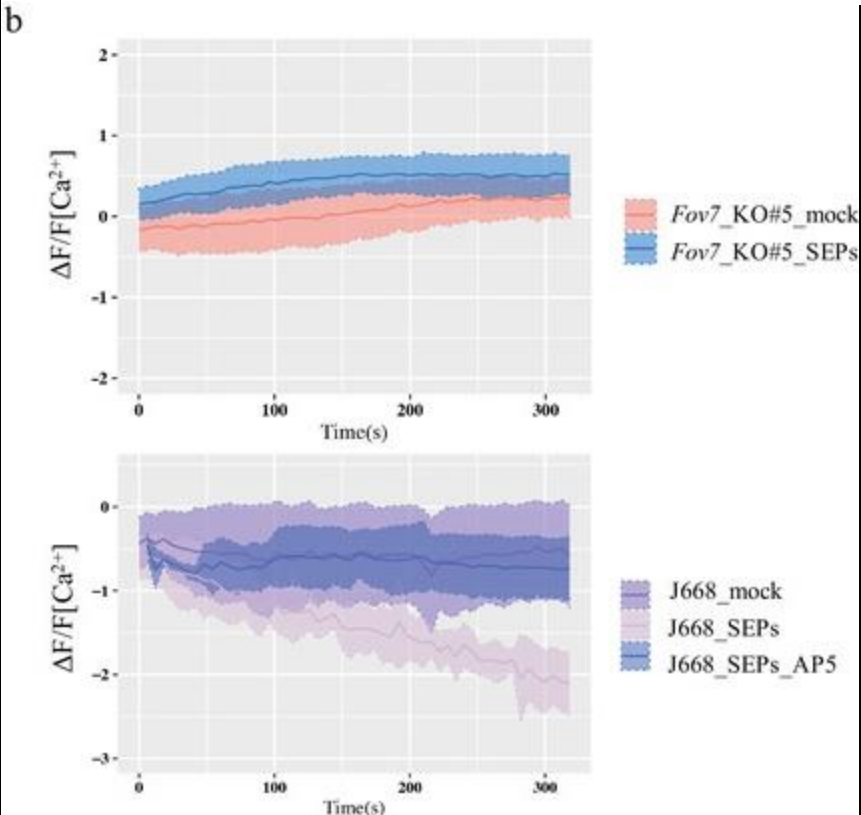
Published online 2021 Feb 19. doi: [10.1002/advs.202002723](https://doi.org/10.1002/advs.202002723)

PMCID: PMC8025038

PMID: [33854882](https://pubmed.ncbi.nlm.nih.gov/33854882/)

A Single-Nucleotide Mutation in a GLUTAMATE RECEPTOR-LIKE Gene Confers Resistance to Fusarium Wilt in *Gossypium hirsutum*

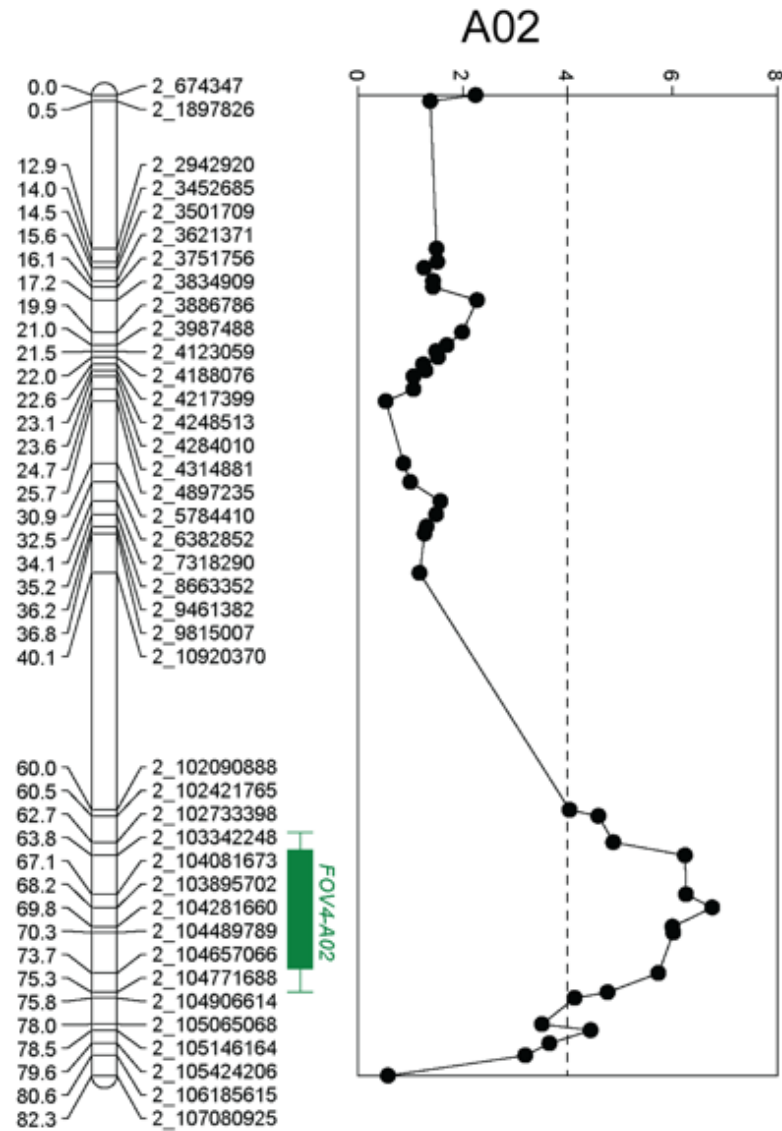
Shiming Liu,¹ Xiaojun Zhang,¹ Shenghua Xiao,¹ Jun Ma,² Weijun Shi,² Tao Qin,¹ Hui Xi,³ Xinhui Nie,³ Chunyuan You,⁴ Zheng Xu,¹ Tianyi Wang,¹ Yujing Wang,¹ Zhennan Zhang,¹ Jianying Li,¹ Jie Kong,² Alifu Aierxi,² Yu Yu,⁵ Keith Lindsey,⁶ Steven J. Klosterman,⁷ Xianlong Zhang,¹ and Longfu Zhu¹



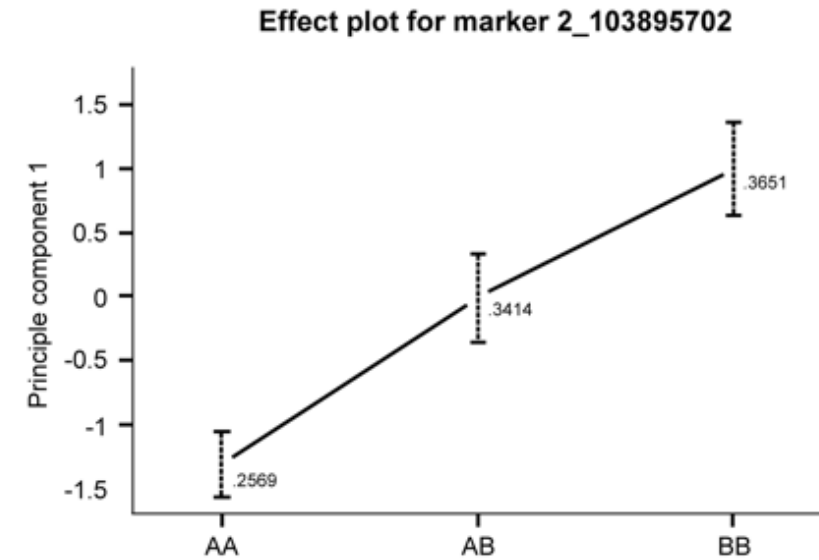
FOV7 in China

D03 GRL contribution to FOV7 and FOV4 resistance and cytosolic calcium influx

A.



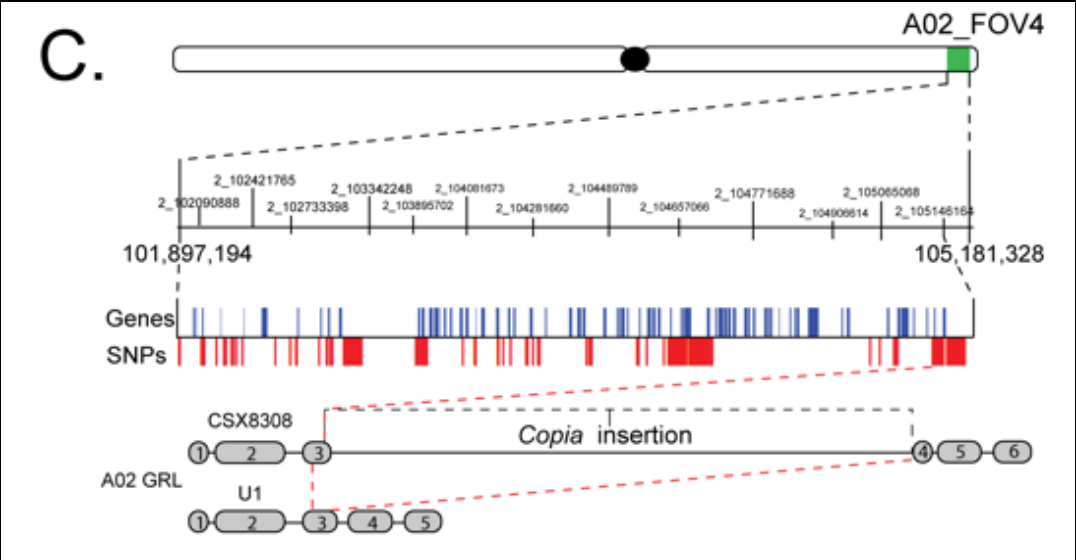
E.



QTL on chromosome A02

3.3 Megabases
163 genes

A02 QTL gene discovery

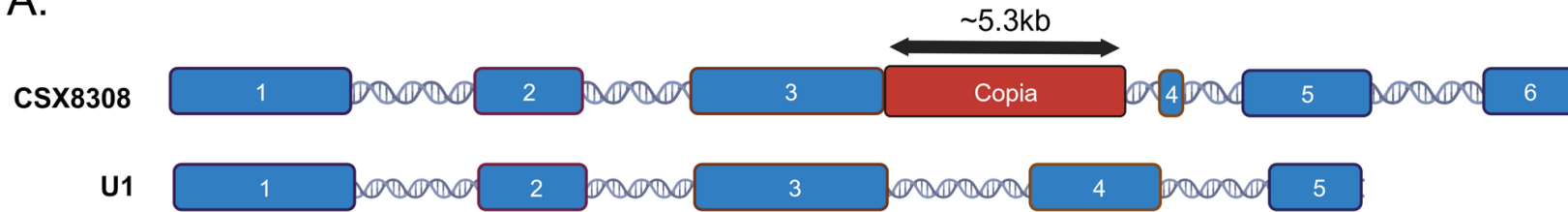


Most Resistant F3s

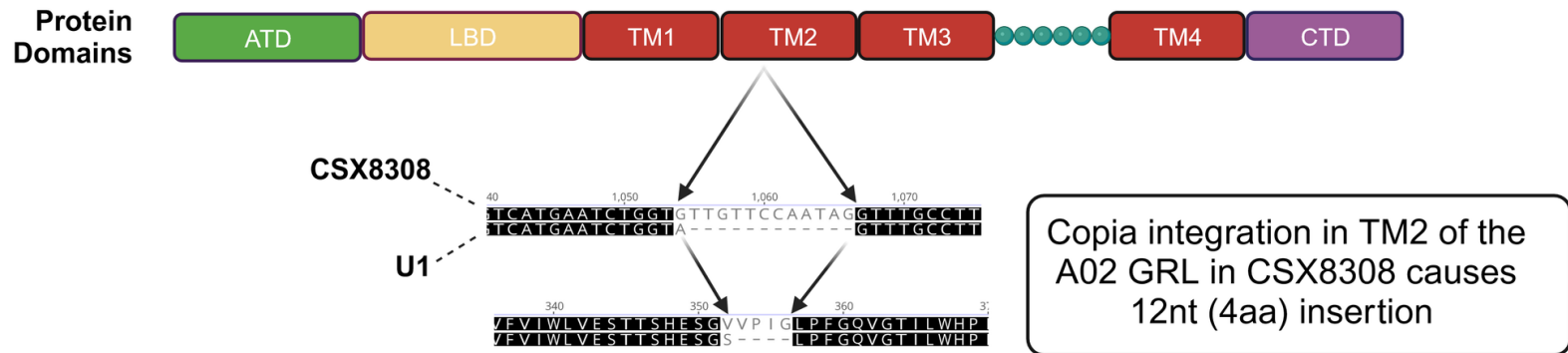
CHROM	POS	REF	ALT	EFF[*].GENE	Gene IPS	CSX8308	U1	DJ8-22	DJ8-65	DJ8-64	DJ8-31	DJ8-66	DJ8-72	DJ8-80	DJ8-57	DJ8-75
A02	102733398	Bin Marker	4.585067		#N/A		--									
A02	102814191	C	T	GhCSX8308.A02G175700	BED zinc finger	BB	AA	AB	AB	BB	./.	./.	AA	AA	AA	AA
A02	102814658	T	C	GhCSX8308.A02G175700	BED zinc finger	BB	AA	AA	AB	BB	./.	AA	./.	AA	AA	AA
A02	102832548	A	G	GhCSX8308.A02G175800	SCO1/SenC	BB	AA	AB	AB	BB	./.	./.	AA	AA	AA	AA
A02	103342248	Bin Marker	4.867675		#N/A		--									
A02	103830254	C	G	GhCSX8308.A02G180300		BB	AA	AB	AA	BB	AB	AA	AA	AA	AA	AA
A02	103830935	G	T	GhCSX8308.A02G180300		BB	AA	AB	AA	BB	AB	AA	AA	AA	AA	AA
A02	103832224	T	G	GhCSX8308.A02G180300		BB	AA	AB	AA	BB	AB	AA	AA	AA	AA	AA
A02	103895702	Bin Marker	6.173494		#N/A		--									
A02	104081673	Bin Marker	6.806055		#N/A		--									
A02	104281660	Bin Marker	6.206119		#N/A		--									
A02	104409537	A	G	GhCSX8308.A02G183400	Domain of unknown function (DUF4228)	BB	AA	AA	AA	BB	./.	AA	AA	AA	AA	AA
A02	104414730	T	C	GhCSX8308.A02G183500	RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain)	BB	AA	AB	AA	BB	AB	AA	AA	AA	AA	AA
A02	104489789	Bin Marker	6.070021		#N/A		--									
A02	104749364	T	C	GhCSX8308.A02G186500		BB	AA	AB	AA	BB	AB	AA	AA	AA	AA	AB
A02	104762907	G	A	GhCSX8308.A02G186600	U-box domain	BB	AA	AA	AA	./.	AB	AA	AA	AA	./.	BB
A02	104762984	C	T	GhCSX8308.A02G186600	U-box domain	BB	AA	AA	AA	./.	AB	AA	AA	AA	AA	BB
A02	104775940	G	A	GhCSX8308.A02G186700	Xylanase inhibitor N-terminal	BB	AA	AB	AA	BB	AB	AA	AA	AA	AA	AB
A02	105116741	T	A	GhCSX8308.A02G189600	Alternative splicing regulator	BB	AA	BB	AA	./.	AA	AA	AA	AA	AA	AB
A02	-----	Del	VVPIG	GhCSX8308.A02G189700	Ligand-gated ion channel	BB	AA	AB	AA	BB	AB	AA	AA	AA	AA	AB
A02	105166646	GWAS	3.291685059			BB	AA	AA	AA	AB	BB	AA	AA	AA	AA	AA
A02	105169427	C	A	GhCSX8308.A02G189800	Protein of unknown function (DUF1685)	BB	AA	AB	AA	BB	AB	AA	AA	AA	AA	AB
A02	105209034	GWAS	3.317285549			BB	AA	AB	AA	AA	AA	./.	AA	AA	AA	AB
A02	105262394	C	G	GhCSX8308.A02G190300	Cytochrome P450	BB	AA	AB	AA	AA	AB	./.	AA	AA	AA	AB
A02	105668784	T	A	GhCSX8308.A02G193200	Reticulon	BB	AA	AB	AA	AA	AB	AA	AA	AA	AA	AB
A02	106741504	G	A	GhCSX8308.A02G203000	Uncharacterised protein family (UPF0183)	BB	AA	AA	AB	AA	AB	AA	AA	AA	AA	BB
A02	106913248	G	T	GhCSX8308.A02G205700	von Willebrand factor type A domain	BB	AA	AA	AA	./.	AA	AA	AA	AA	./.	BB

Transposon insertion disrupts gene function

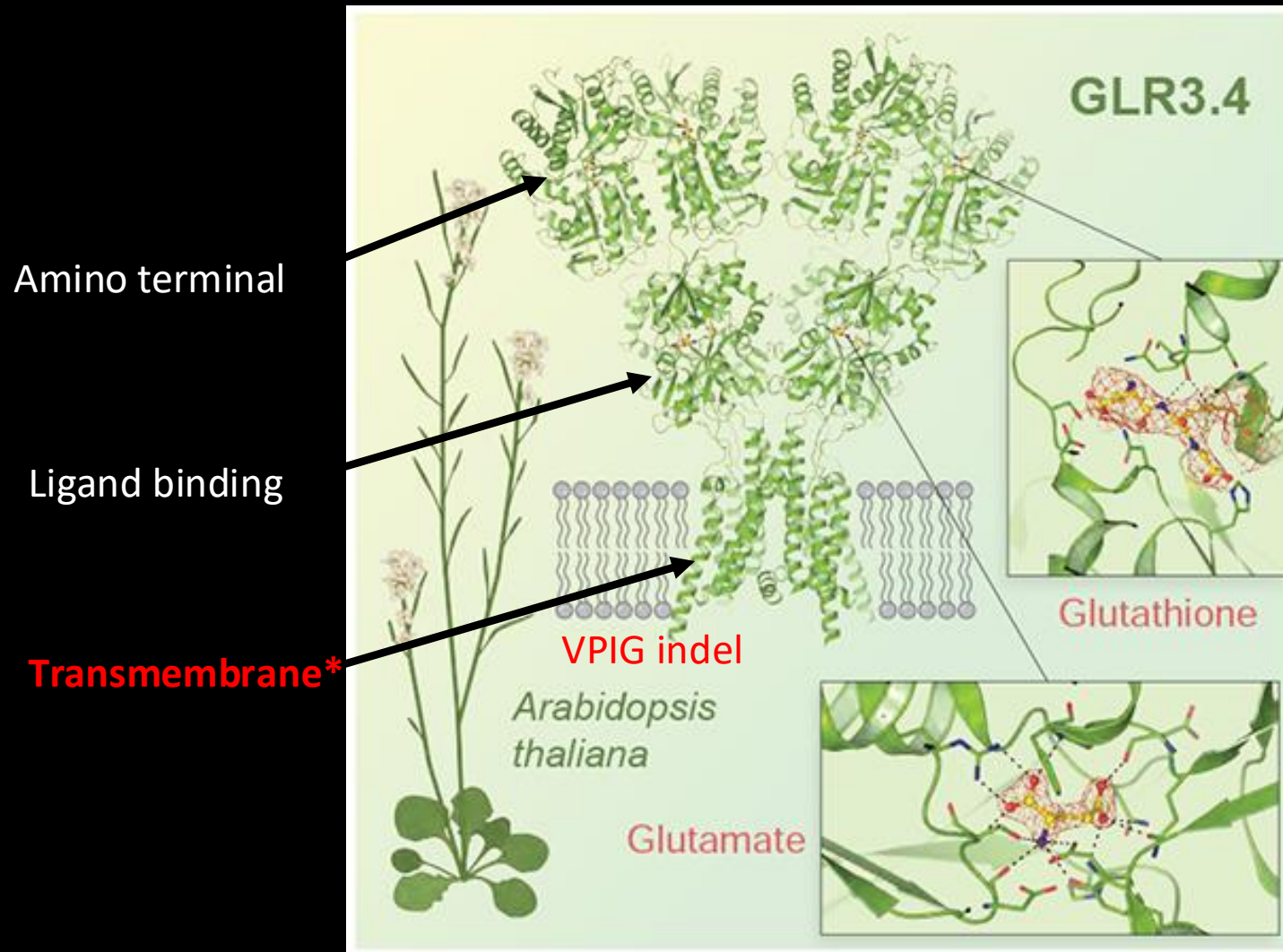
A.



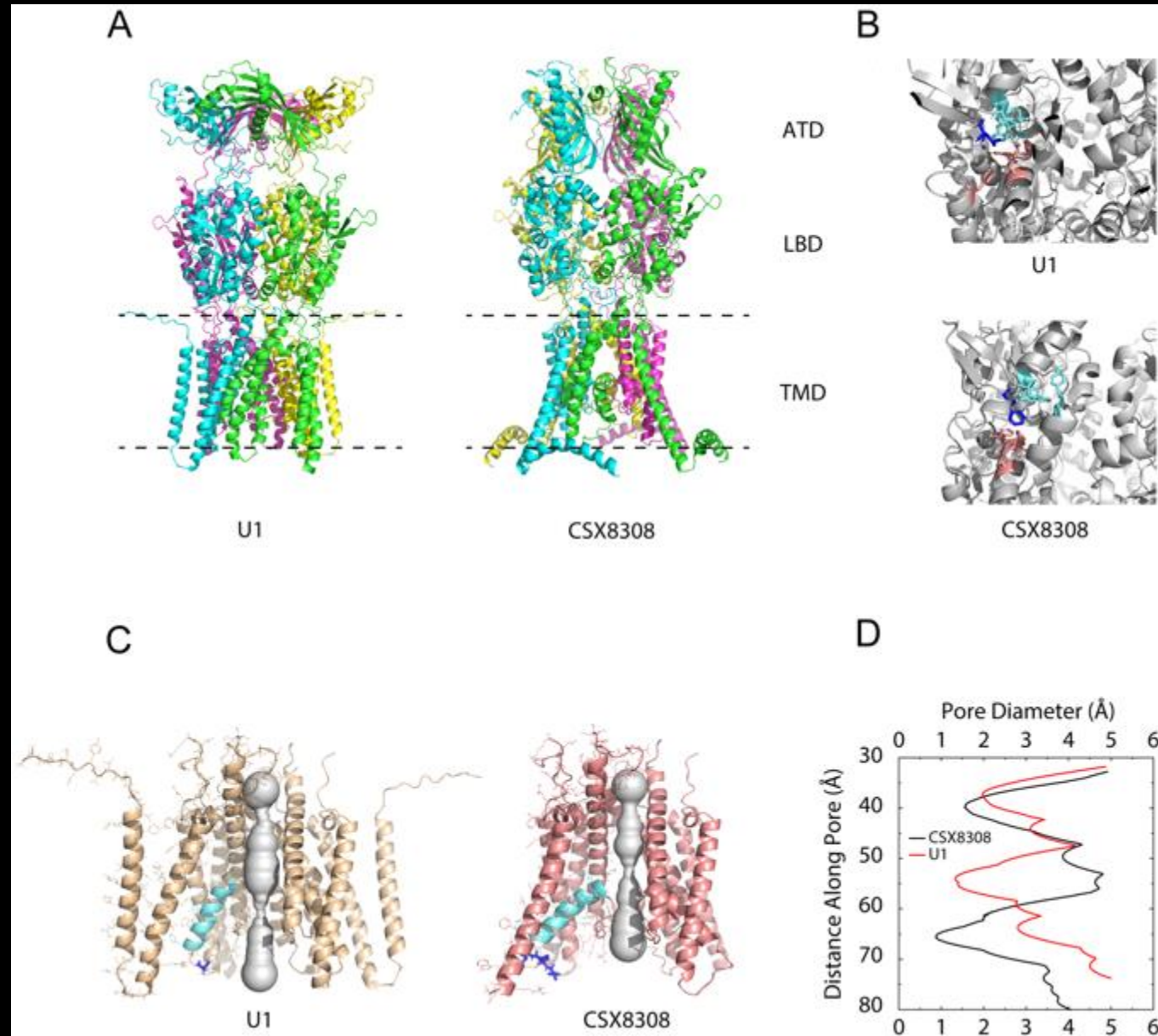
B.



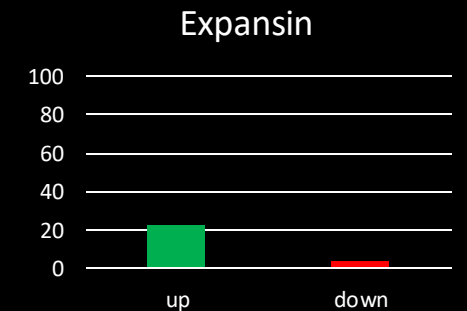
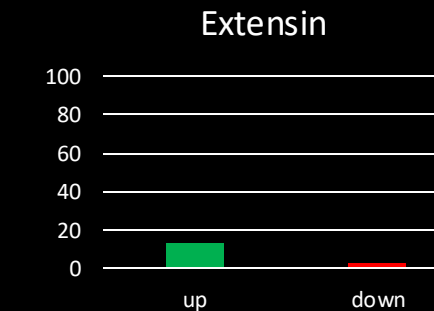
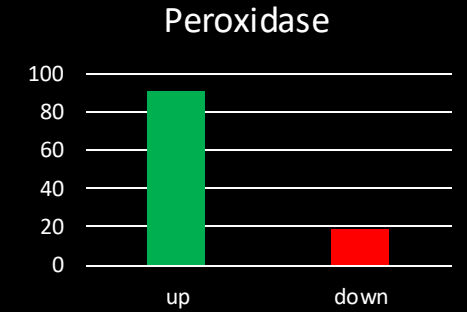
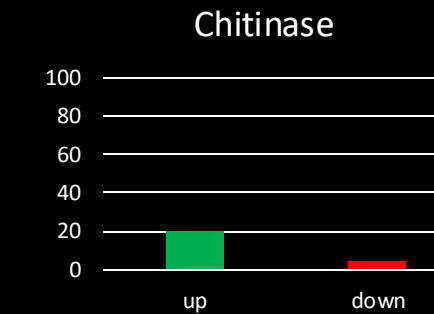
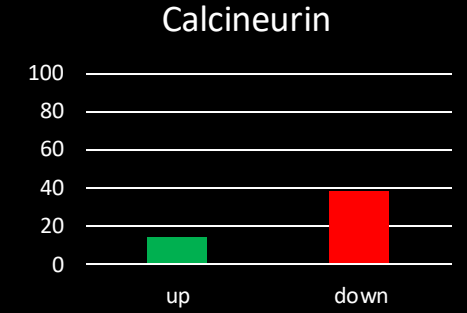
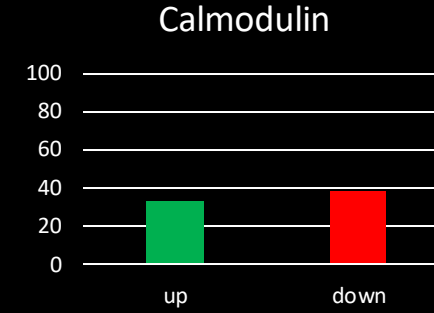
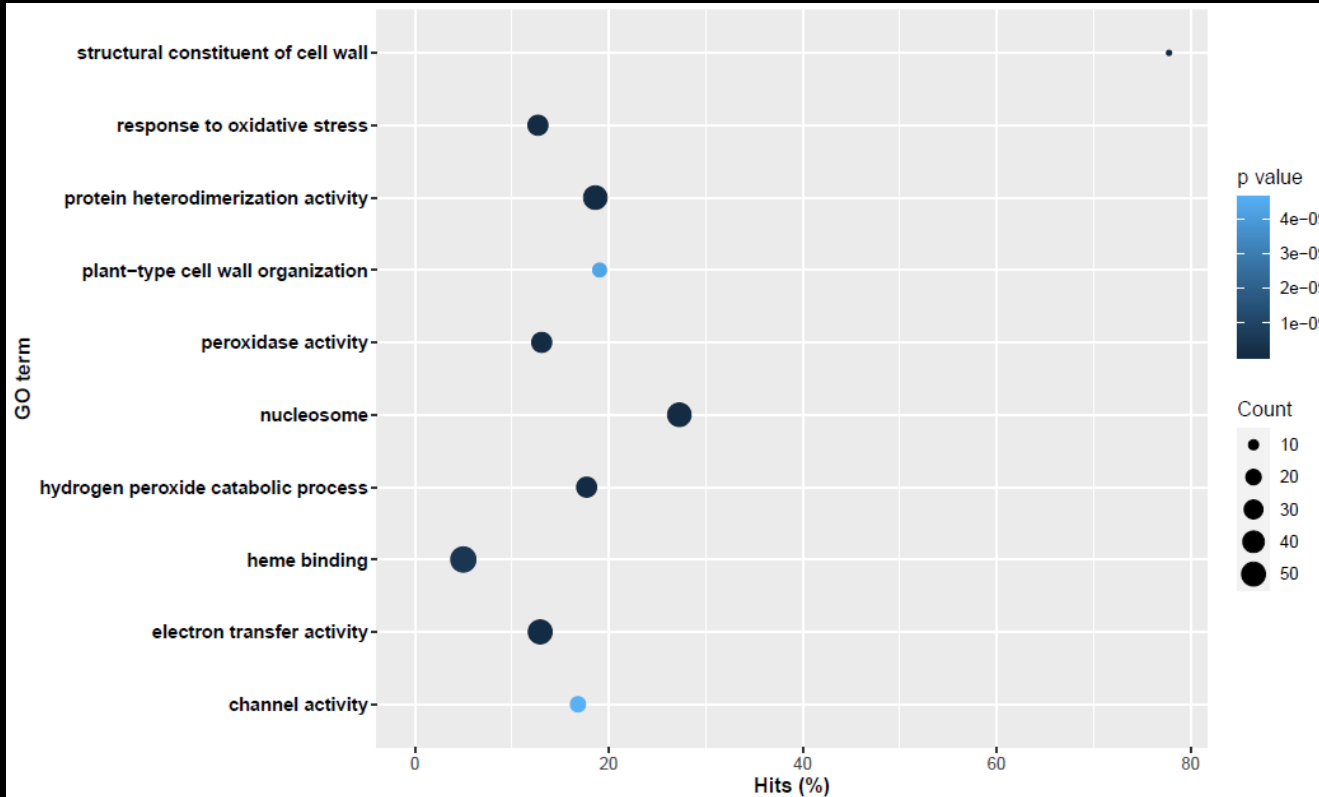
Plant glutamate receptors



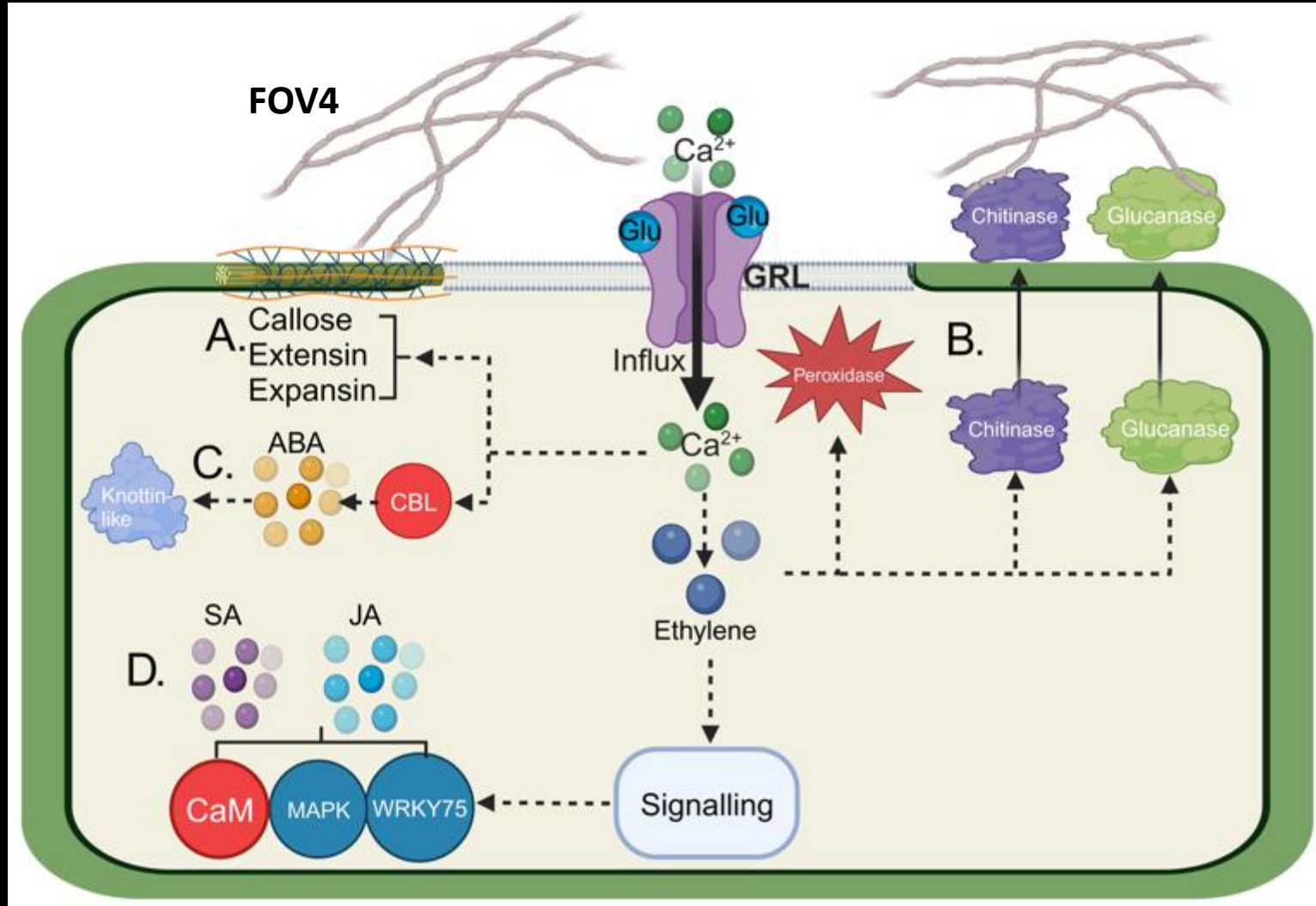
A02 GLR pore size in U1 is larger



Transcriptomics underlying FOV4 resistance in 'U1'



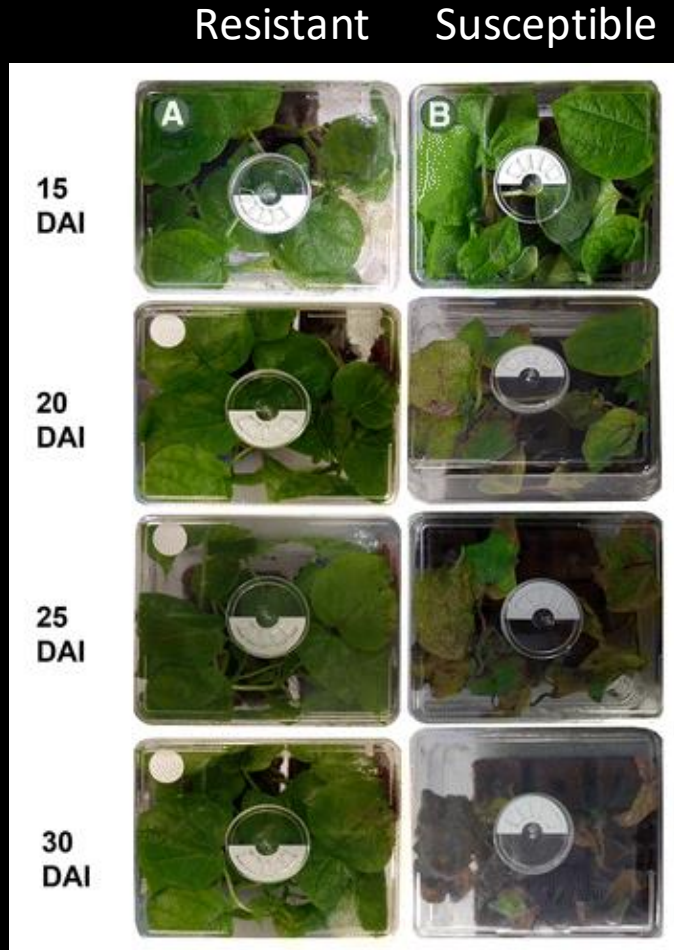
Potential GRL-mediated mechanisms for FOV4 resistance



Summary

- Geostatistics improves field phenotyping.
- Early generational genetic mapping enhanced by whole genome sequencing can expedite causal gene discovery
- GLRs are a potential new class of R-gene?
- Volumetric calcium influx catalyzes robust defense responses

RNAseq analysis of FOV4 challenge in pima



Collected tissue at 3 time points:

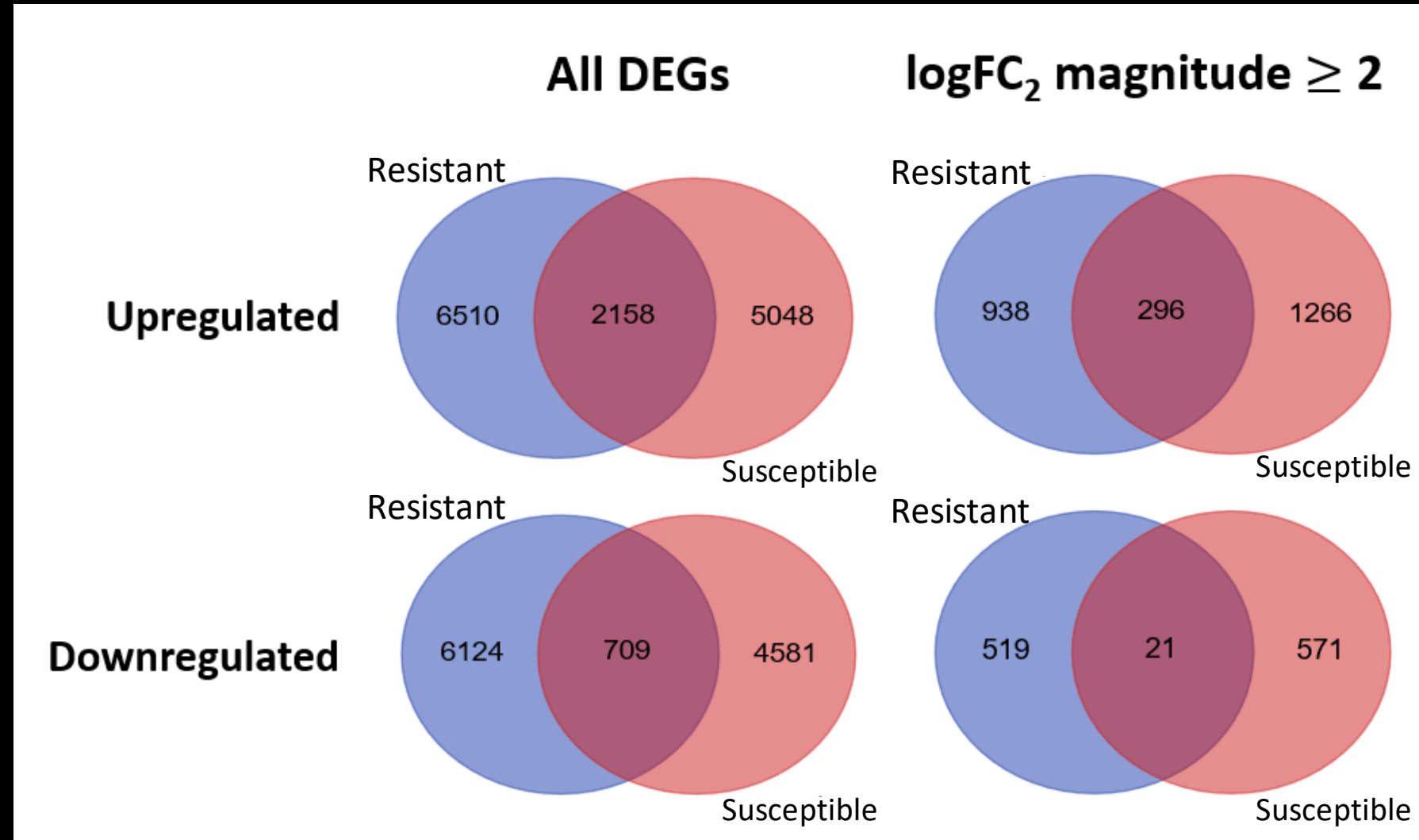
12hai, 24hai, 72hai

Extract RNA for RNAseq

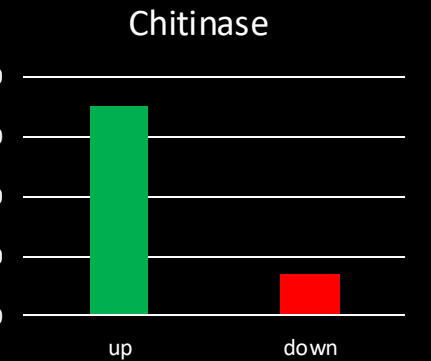
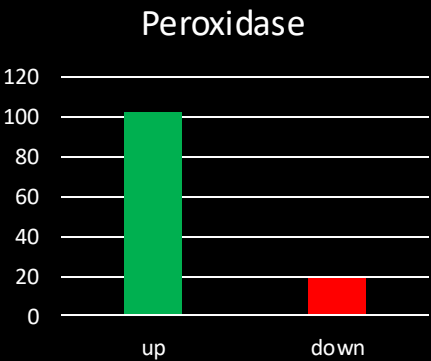
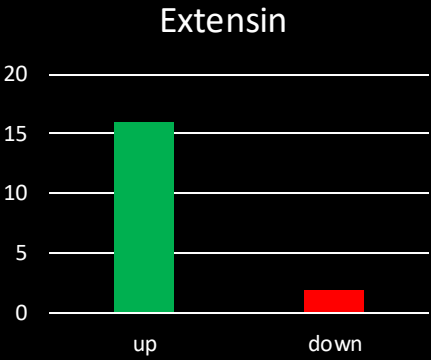
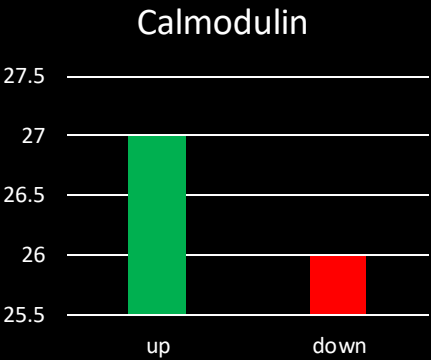
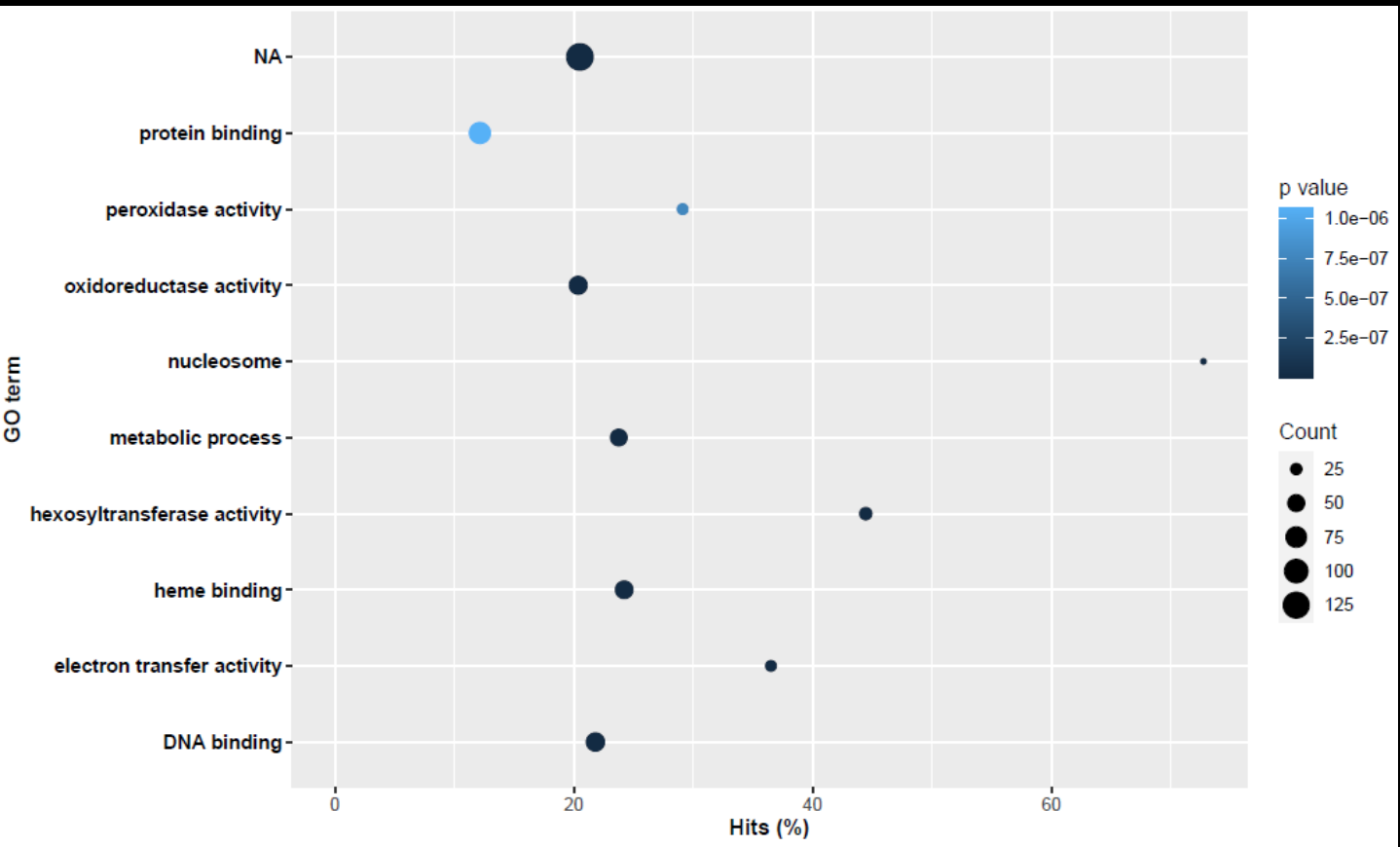
Identify unique expression in resistant pima



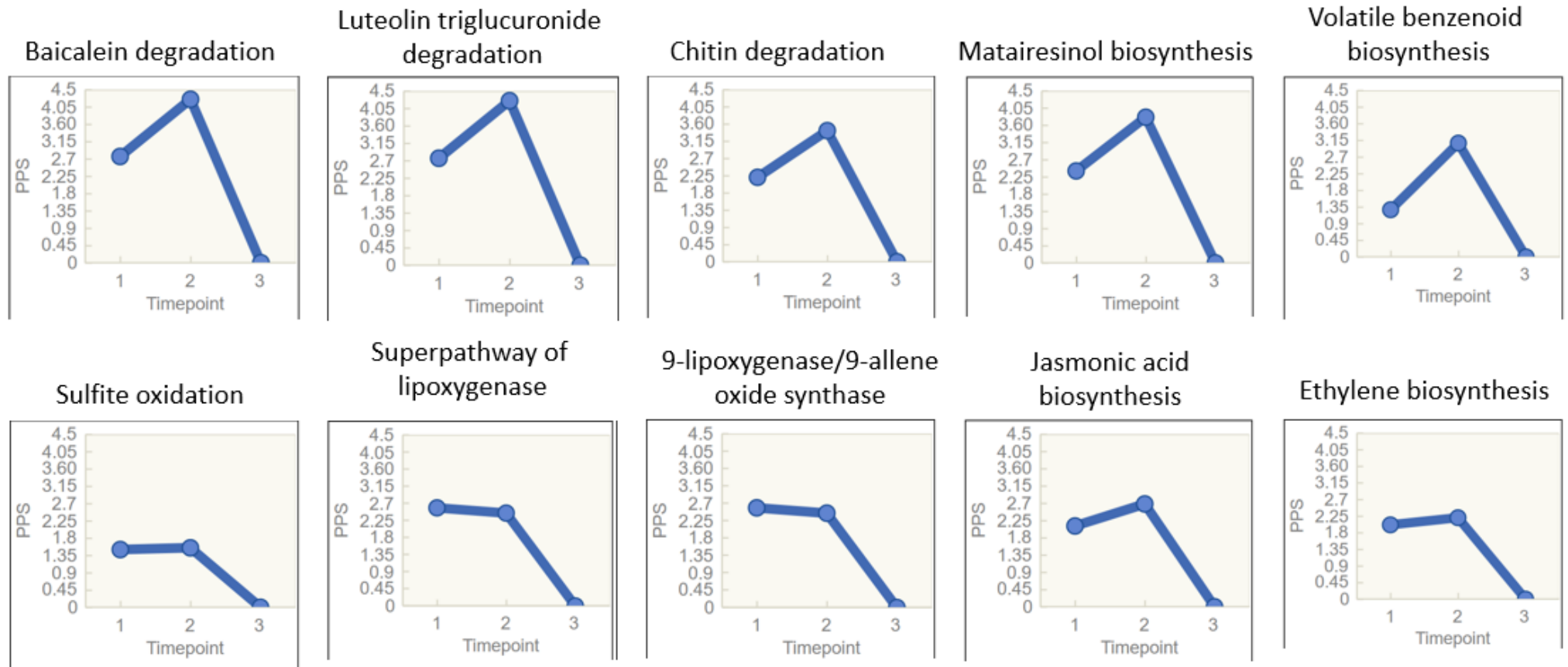
Induced gene counts



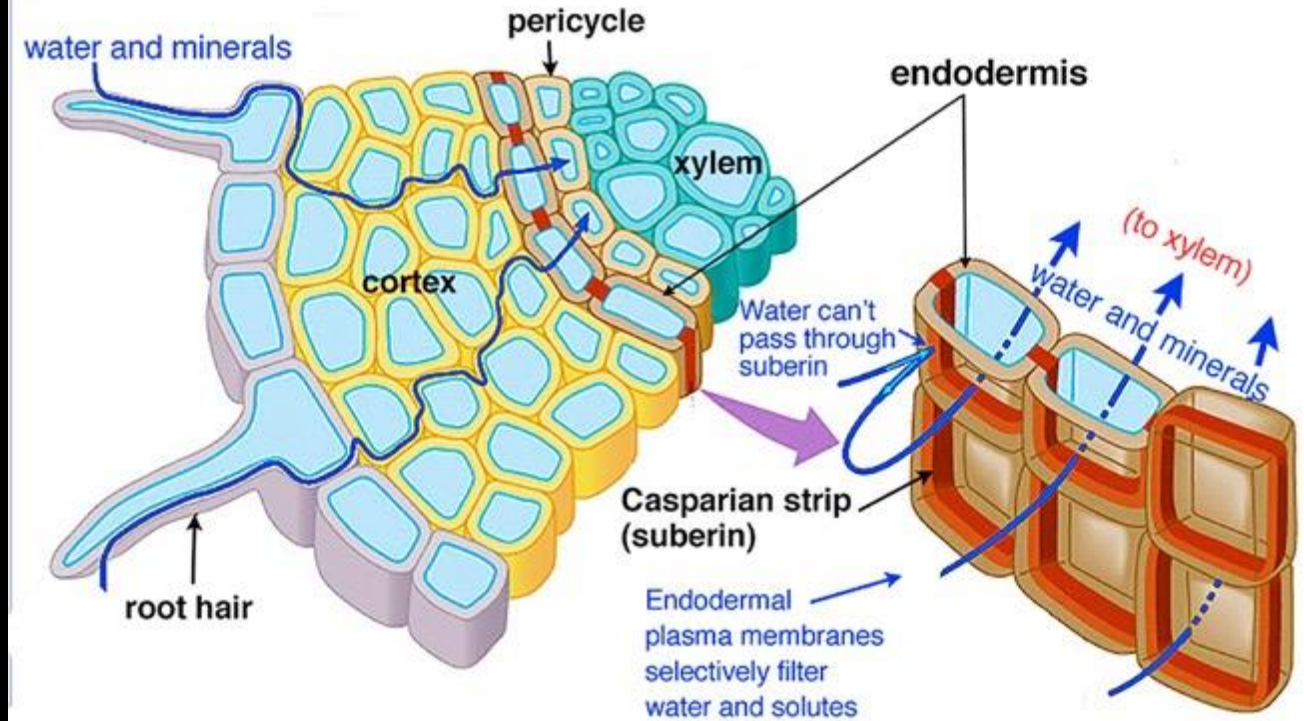
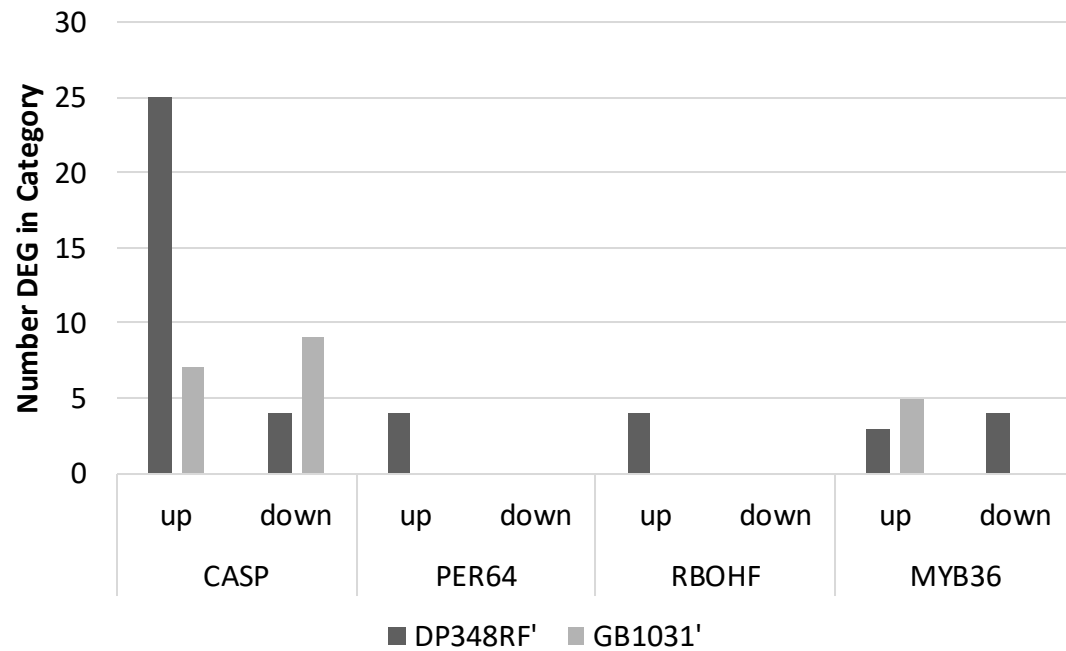
GO enrichment



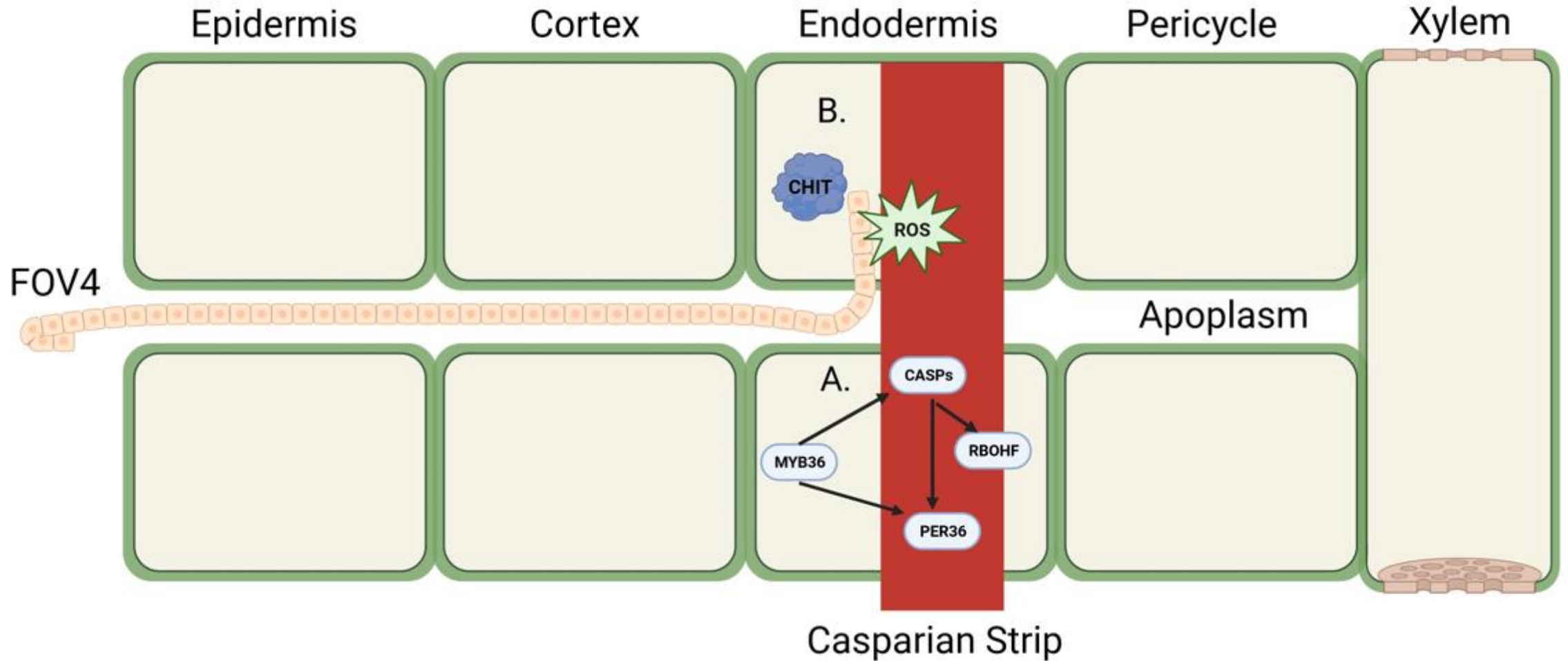
Biochemical pathway enrichment



Casparian strip-associated DEG abundance



Potential role of the Casparian strip in the restriction of vascular colonization



Conclusions

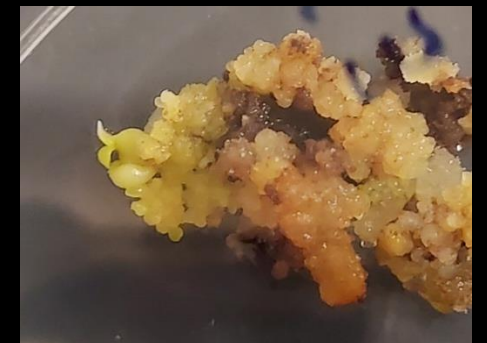
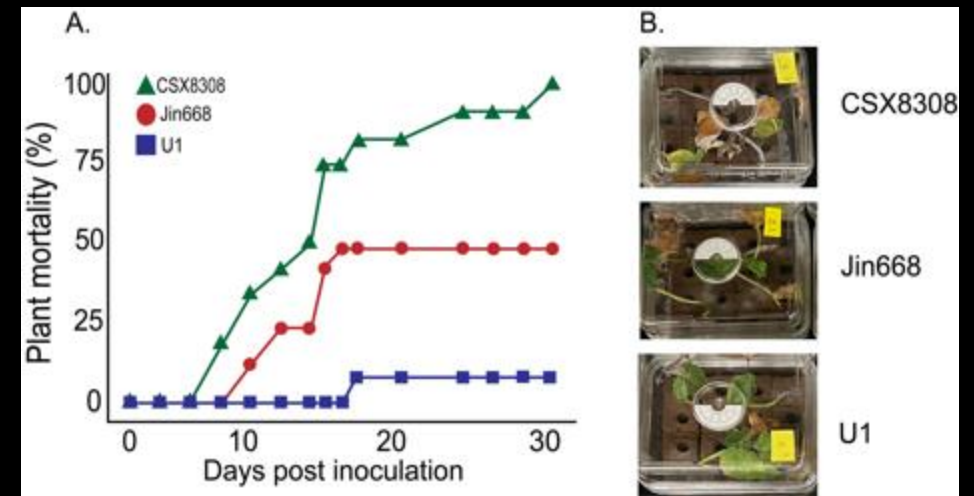
- Reactive oxidative bursts underly FOV4 resistance in both species
- Wall off mechanisms play pivotal role in FOV4 resistance in both upland and pima
 - General cell wall fortification
 - Casparian strip fortification provides a biochemical barrier against vascular pathogen invasion
- GLRs are new targets for investigation with providing broad spectrum fungal resistance
 - Targets for transformation
- Calcium signaling



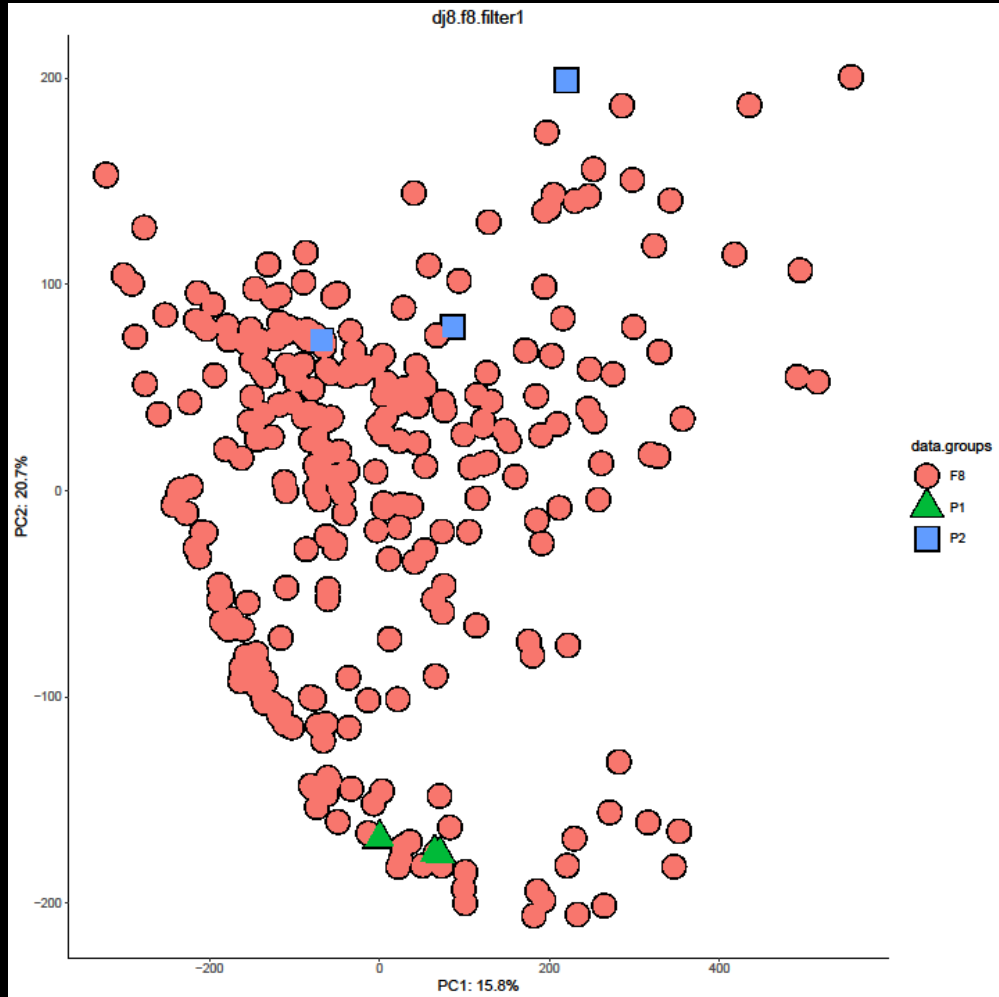
Ongoing Work

Gene Validation

1. Knock-in of A02 GRL in Jin668 (D03 exists naturally)
2. Knock-out of D03 in Jin668
3. Knock-in of A02 in Coker312 & KO of endogenous
4. Knock-in of A02 & D03 in Coker312 & KO of endogenous
5. Direct editing in U1 (regeneration protocol optimization)



RIL Genotypes (Stephen graphs)



- F8 RIL population (U1 x CSX8308)
- Phenotyped in El Paso, Fall 2023
- Skim sequenced 3-5X (HA)
- >5k quality SNPS



Cotton Cross Section Stain Analysis - Computer Vision

DJ-8-110

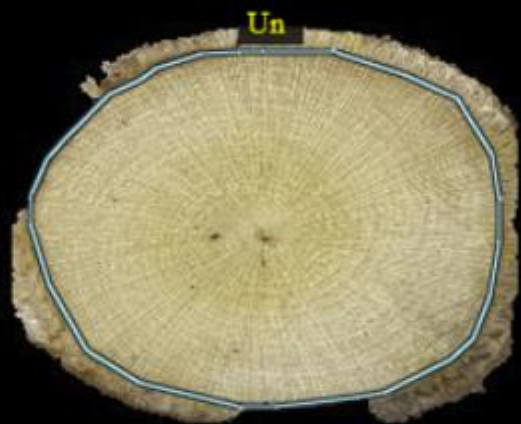
Total vascular tissue area: 13351.5 (pixels)

Total stained area: 0

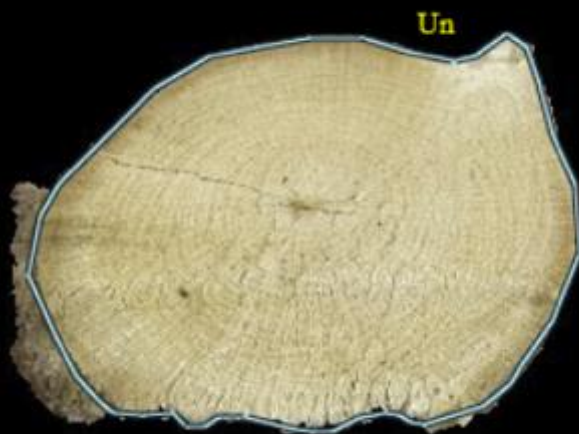
Percent area stained: 0%



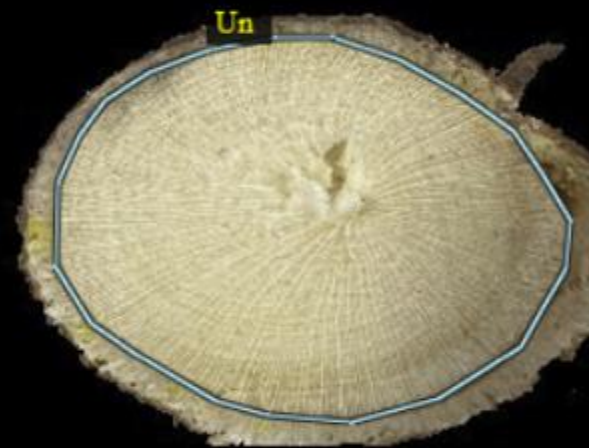
0% Stain Examples



DJ-8-165



DJ-8-113

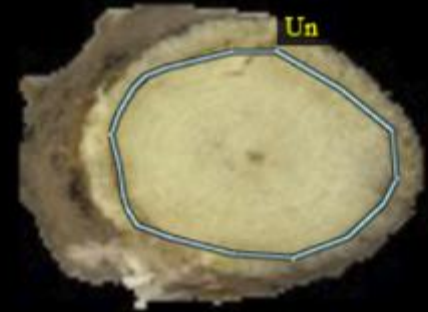


DJ-8-115

Total Surface Area Variability

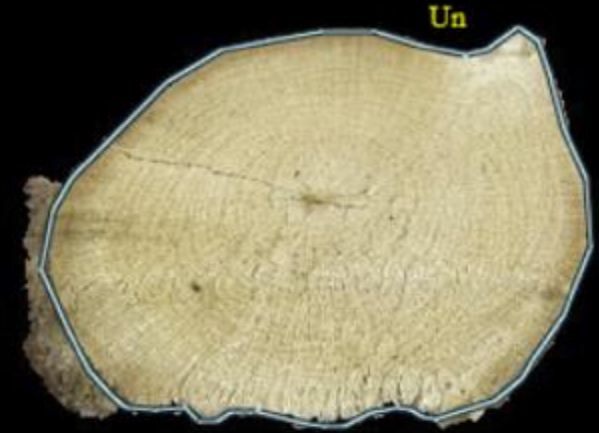
DJ-8-77

Total vascular tissue area: 2895.5



DJ-8-113

Total vascular tissue area: 33235.5



0% Stained Group (All Individuals)

- DJ-8-110
- DJ-8-113
- DJ-8-115
- DJ-8-118
- DJ-8-145
- DJ-8-148
- DJ-8-15
- DJ-8-151
- DJ-8-143
- DJ-8-155
- DJ-8-163
- DJ-8-165
- DJ-8-173
- DJ-8-175
- DJ-8-181
- DJ-8-193
- DJ-8-207
- DJ-8-216
- DJ-8-217
- DJ-8-222
- DJ-8-236
- DJ-8-240
- DJ-8-246
- DJ-8-260
- DJ-8-266
- DJ-8-268
- DJ-8-278
- DJ-8-283
- DJ-8-34
- DJ-8-43
- DJ-8-56
- DJ-8-57
- DJ-8-59
- DJ-8-61
- DJ-8-63
- DJ-8-71
- DJ-8-72
- DJ-8-75
- DJ-8-76
- DJ-8-77
- DJ-8-80
- DJ-8-87
- DJ-8-89
- DJ-8-95

44 Individuals (~16%) of 282

High Stain Concentration

DJ-8-99

Total vascular tissue area: 11593

Total stained area: 1804.5

Percent area stained: 15.57%



High Stain Examples cont.

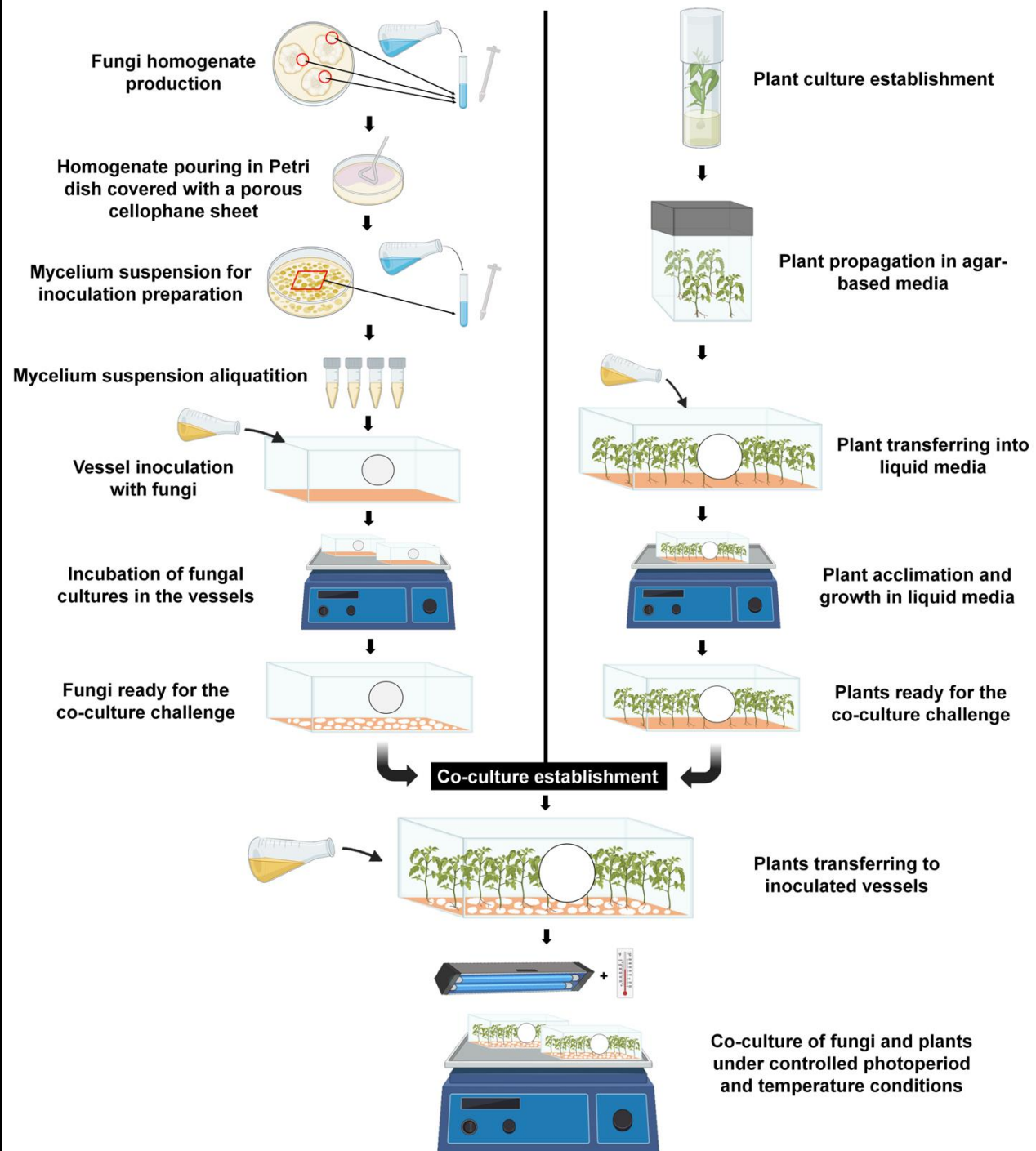
DJ-8-65

Total vascular tissue area: 7328

Total stained area: 4823

Percent area stained: 65.82%





Acknowledgements

Collaborators:

Dr. Don Jones (Cotton Inc)
Dr. Stephen Parris (Clemson)
Dr. Feng Ding (Clemson)
Dr. William Bridges (Clemson)
Dr. Whitney Wang (Clemson)
Dr. Steve Jeffers (Clemson)
Dr. John Lovell (HudsonAlpha)
Dr. Avinash Sreedasyam (HudsonAlpha)
Jeremy Schmutz (HudsonAlpha)
Dr. Warwick Stiller (CSIRO)
Dr. Iain Wilson (CSIRO)

Funding:

Cotton Inc PhD Fellowship (2019-2023)
USDA-NIFA (#2019-67029)
USDA-NIFA (#2021-11395)

O&A Enterprises Inc.



**Cotton
Incorporated**



**FOUNDATION FOR
FOOD & AGRICULTURE
RESEARCH**

