FOV4 Developments

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2024 Breeders Tour

Overview of the Systems Genetics Lab

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

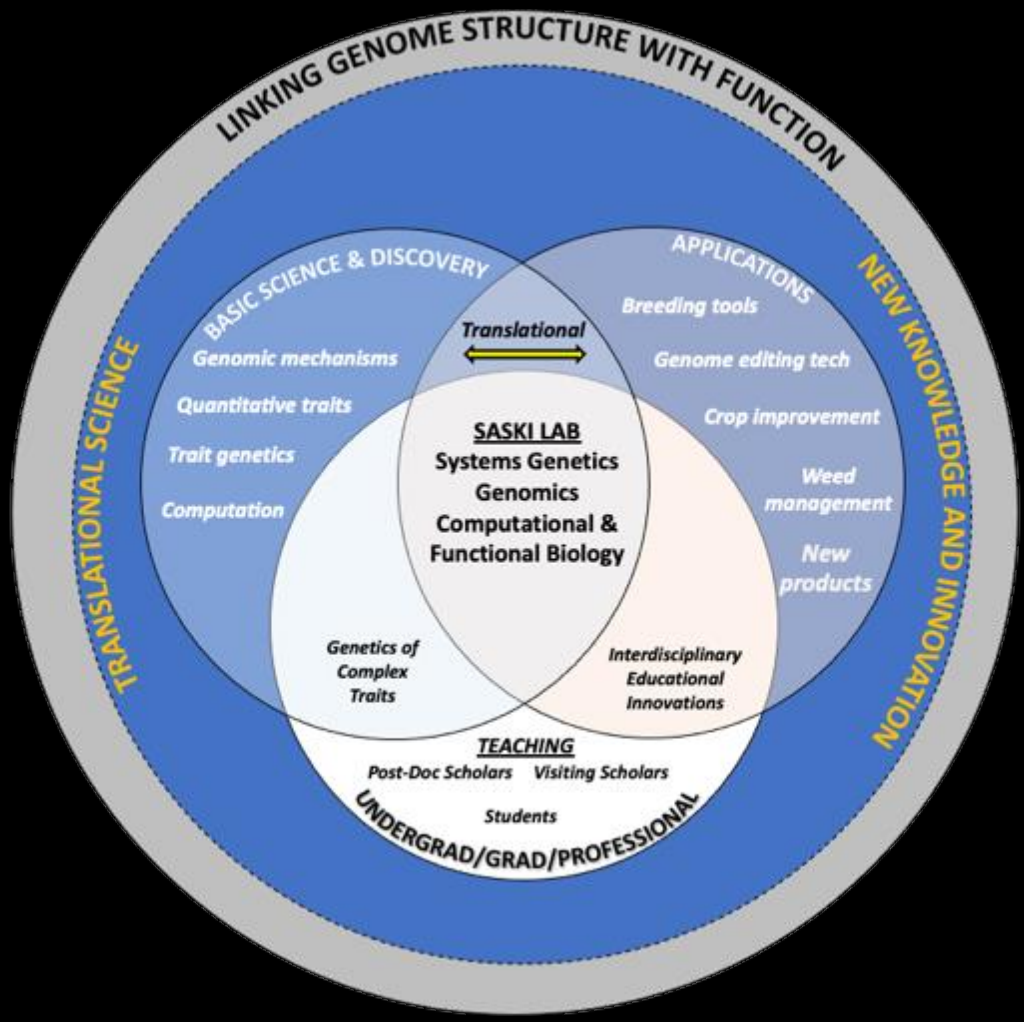
BASIC RESEARCH – (Disciplinary domains: *Genomics, Genetics, Computational Biology, Genome Engineering, Evolutionary Genomics, Synthetic Biology*)

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

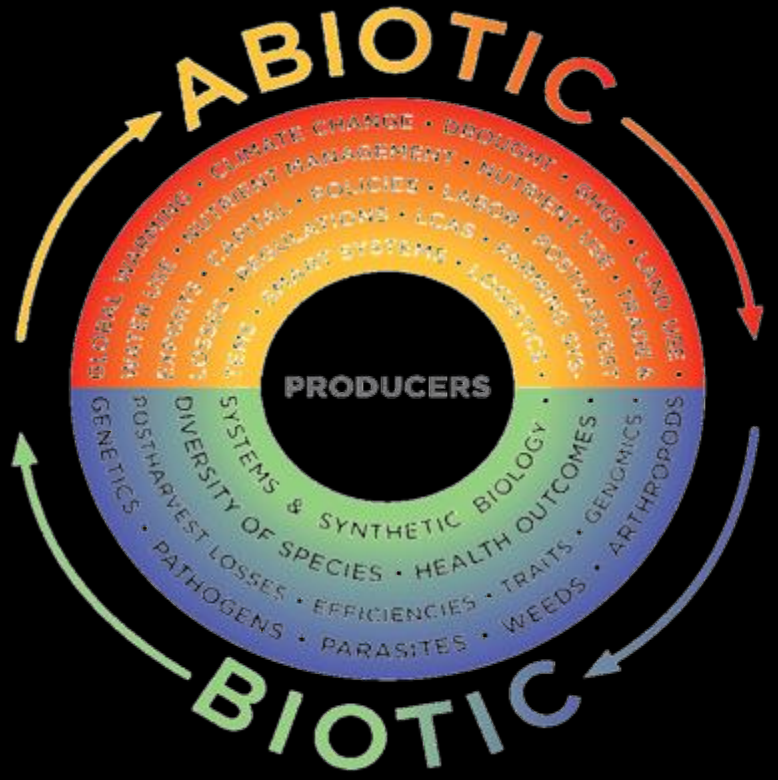
Biotic resilience
Developmental genetics
Fiber traits
Advanced breeding

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

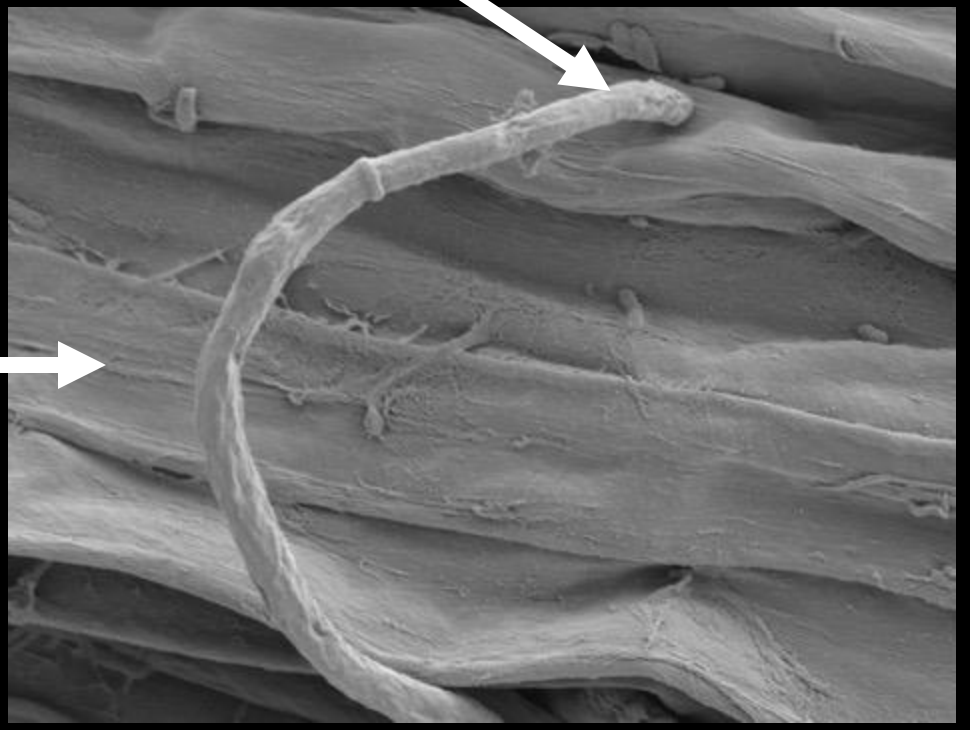
Fusarium wilt in the US

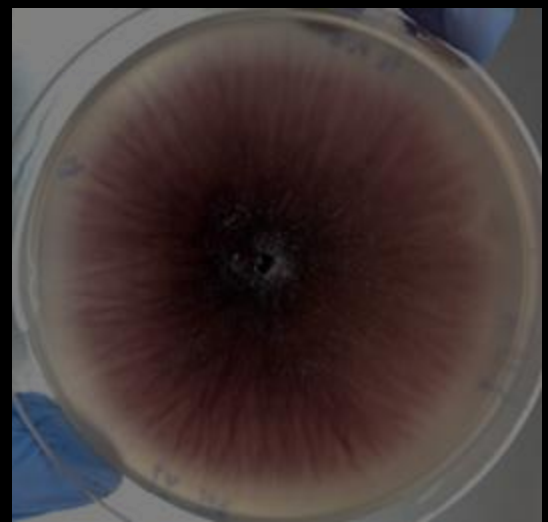
- ✓ *Fusarium oxysporum* f. sp. *vasinfectum*
- ✓ First ID 1892 in Alabama
- ✓ Inoculum dependent
- ✓ No nematodes, no symptoms

Davis et al. 2006

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





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

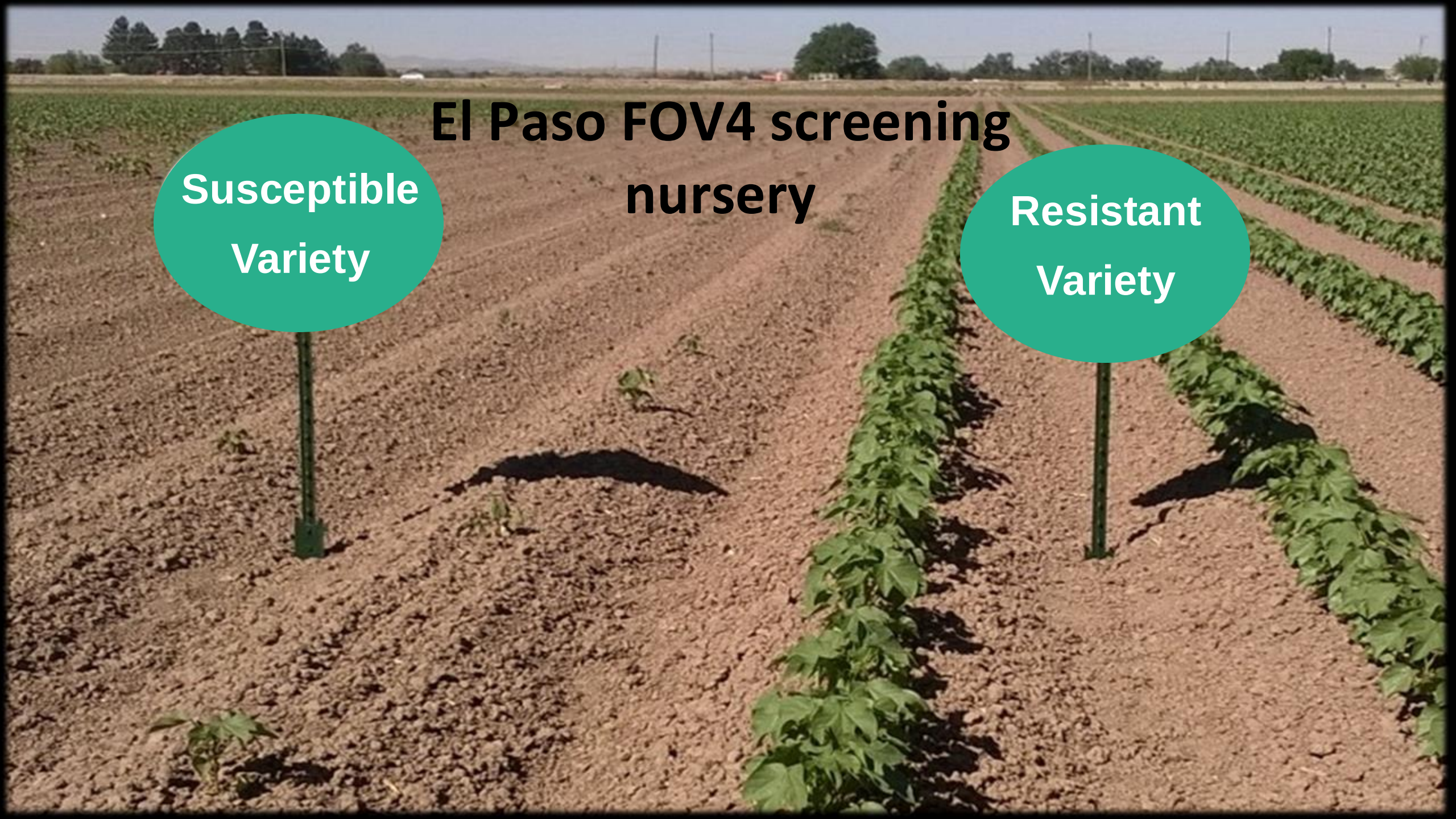
https://www.sjvqualitycotton.com/

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


WYOMING
Chicago
CT
IOWA
PENN
San Francisco
NEVADA
NEBRASKA
New York
ILLINOIS
OHIO
UTAH
United States
INDIANA
Washington
CALIFORNIA
KANSAS
MISSOURI
VIRGINIA
Las Vegas
KENTUCKY
Los Angeles
NORTH CAROLINA
TENNESSEE
San Diego
ARIZONA
OKLAHOMA
Clint
SOUTH CAROLINA
ARKANSAS
MISSISSIPPI
Dallas
ALABAMA
GEORGIA
TEXAS
LOUISIANA
Gulf of California
San Antonio
Houston
FLORIDA
Monterrey
Miami
Gulf of Mexico
Santo Domingo
Mexico
Havana
Cuba
Guadalajara
Mexico City


El Paso FOV4 screening nursery
Susceptible Variety
Resistant Variety

Robust & unique field design

	Row 37		Row 38		Row 39		Row 40		Row 41		Row 42		Row 43		Row 44	
56	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
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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
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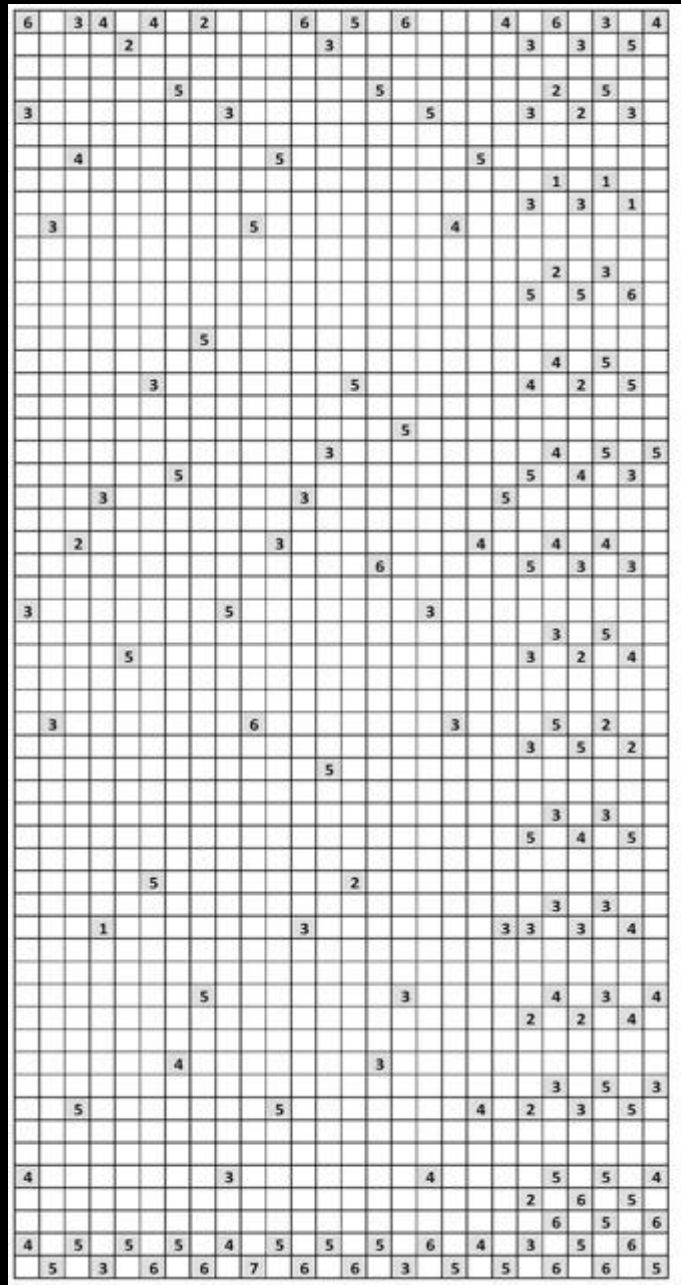
	Row 37		Row 38		Row 39		Row 40		Row 41		Row 42		Row 43		Row 44	



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

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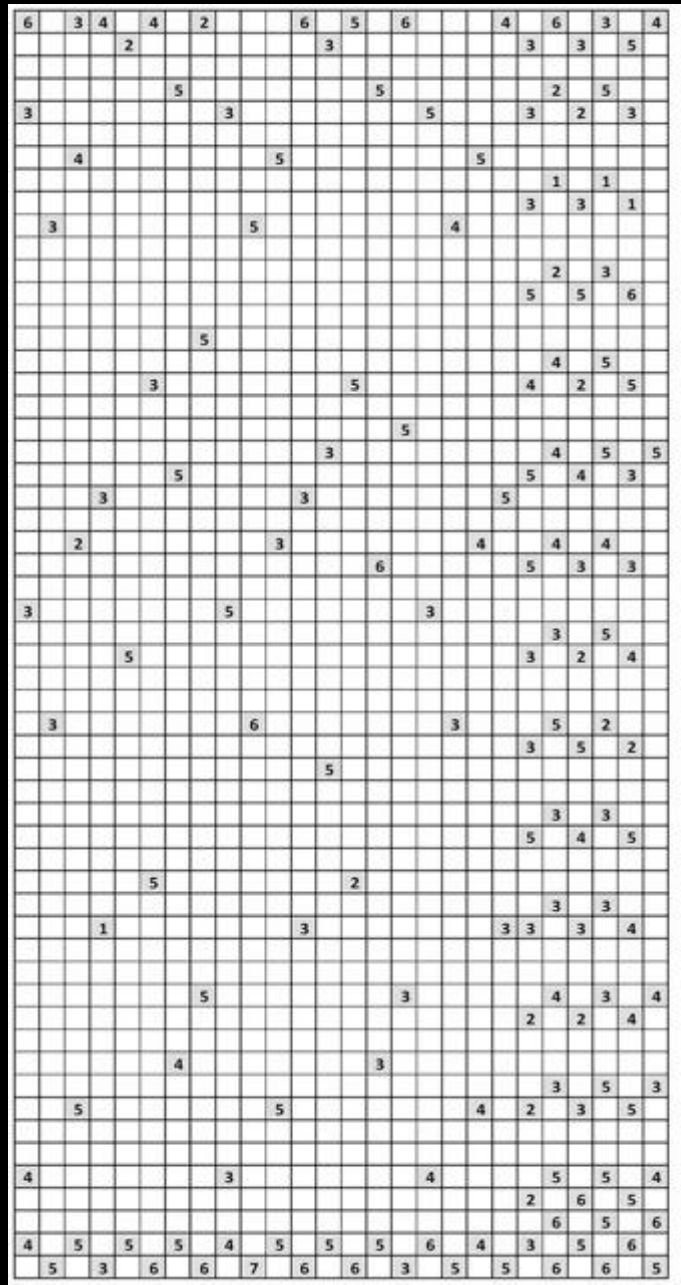
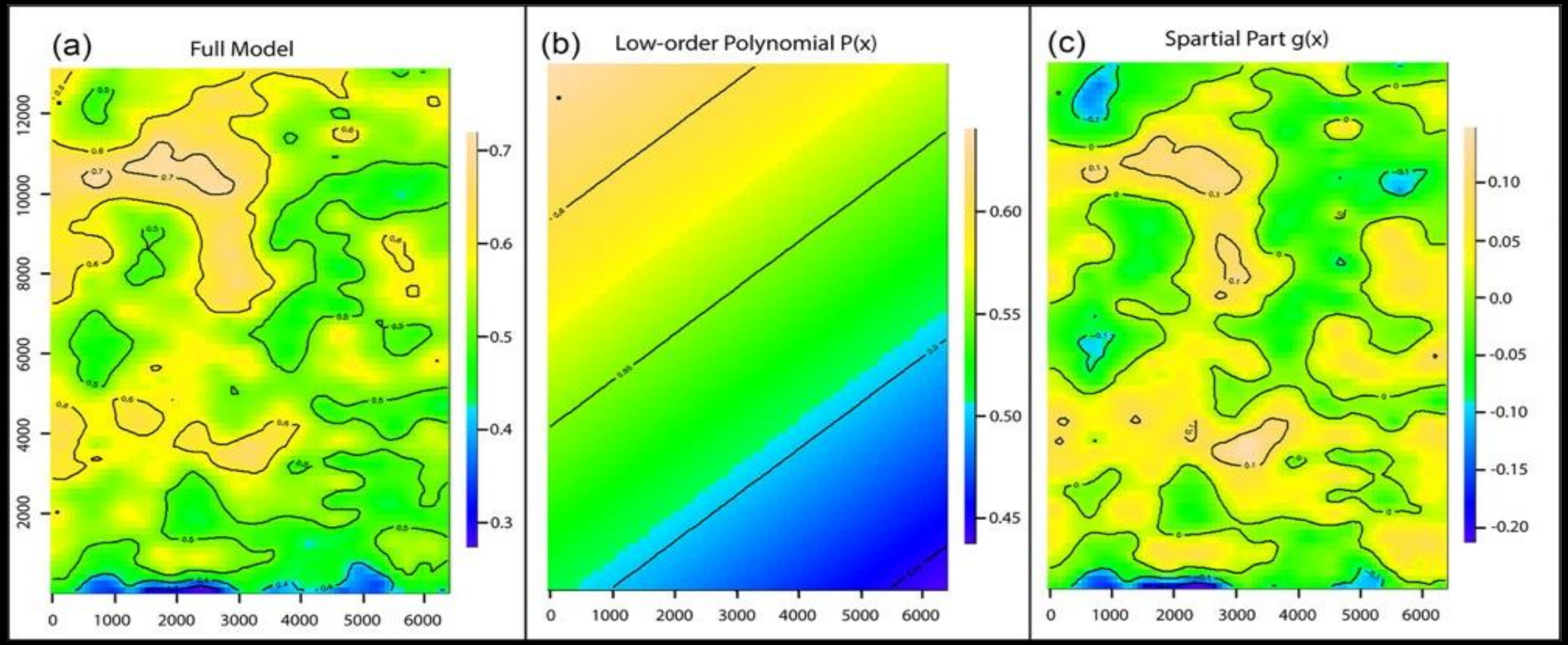
11	DJ-6-5		DJ-6-54		DJ-6-102		DJ-6-150		DJ-6-196		DJ-6-244		DJ-8-4		DJ-8-49	11
10	DJ-6-4		DJ-6-53		DJ-6-101		DJ-6-149		DJ-6-195		DJ-6-243		Mod		DJ-8-48	10
9	DJ-6-3		DJ-6-52		DJ-6-100		DJ-6-148		DJ-6-194		Tol		DJ-8-3		DJ-8-47	9
8	DJ-6-2		DJ-6-51		Mod		DJ-6-147		DJ-6-193		DJ-6-242		DJ-8-2		DJ-8-46	8
7	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	



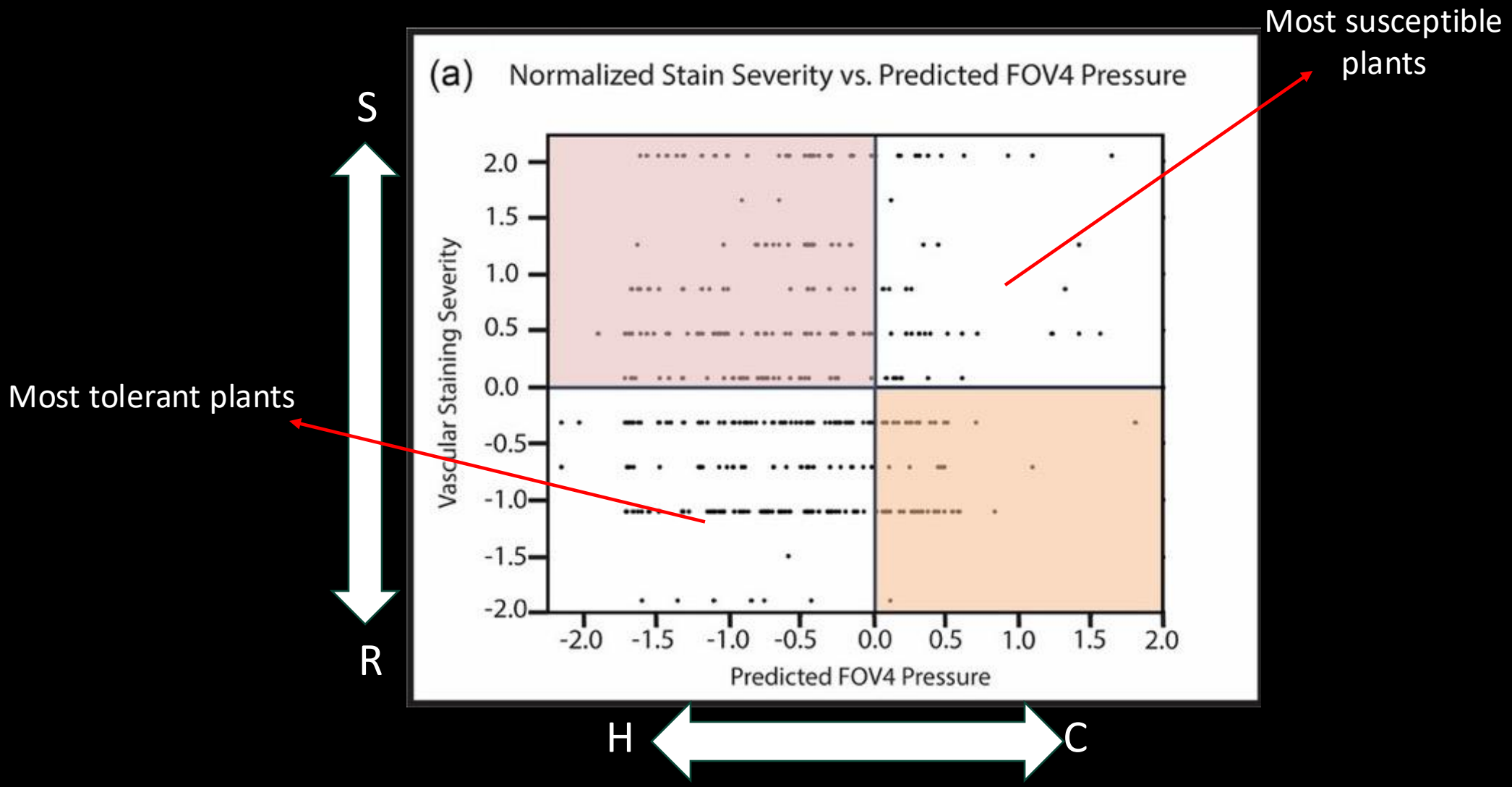
Rating	Plant Survival
0	0%
1	1-10%
2	11-20%
3	21-30%
4	31-40%
5	41-50%
6	51-60%
7	61-70%
8	71-80%
9	81-90%
10	91-100%

Kriging interpolation



Parris et al. (2023) Crop Science, https://doi.org/10.1002/csc2.20961

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



INFORMED SELECTION

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

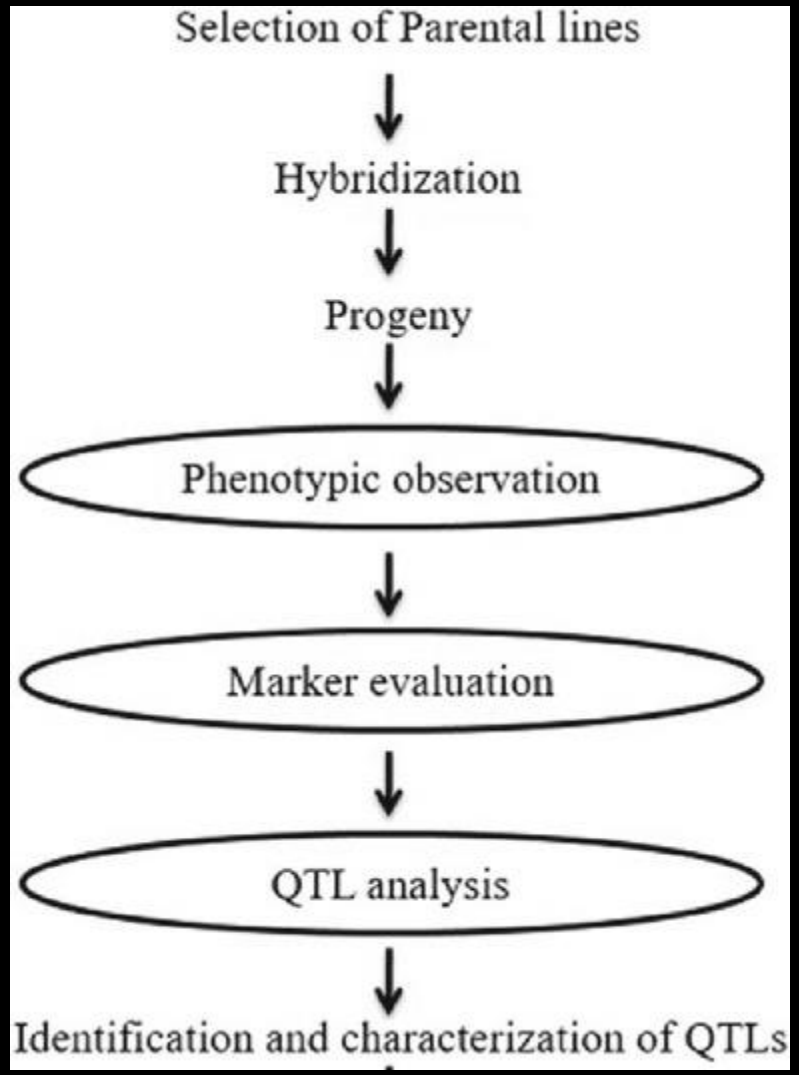
‘CSX8308’ × ‘U1’

288+ F3 individuals

Selection of 94 individual DJ8 plants by inoculum adjusted phenotype

- 1/3 Resistant
- 1/3 Moderate
- 1/3 Susceptible

FOV4 QTL discovery



Reference grade whole genome sequences

Adjusted with inoculum distribution info

Whole genome sequencing of F3s

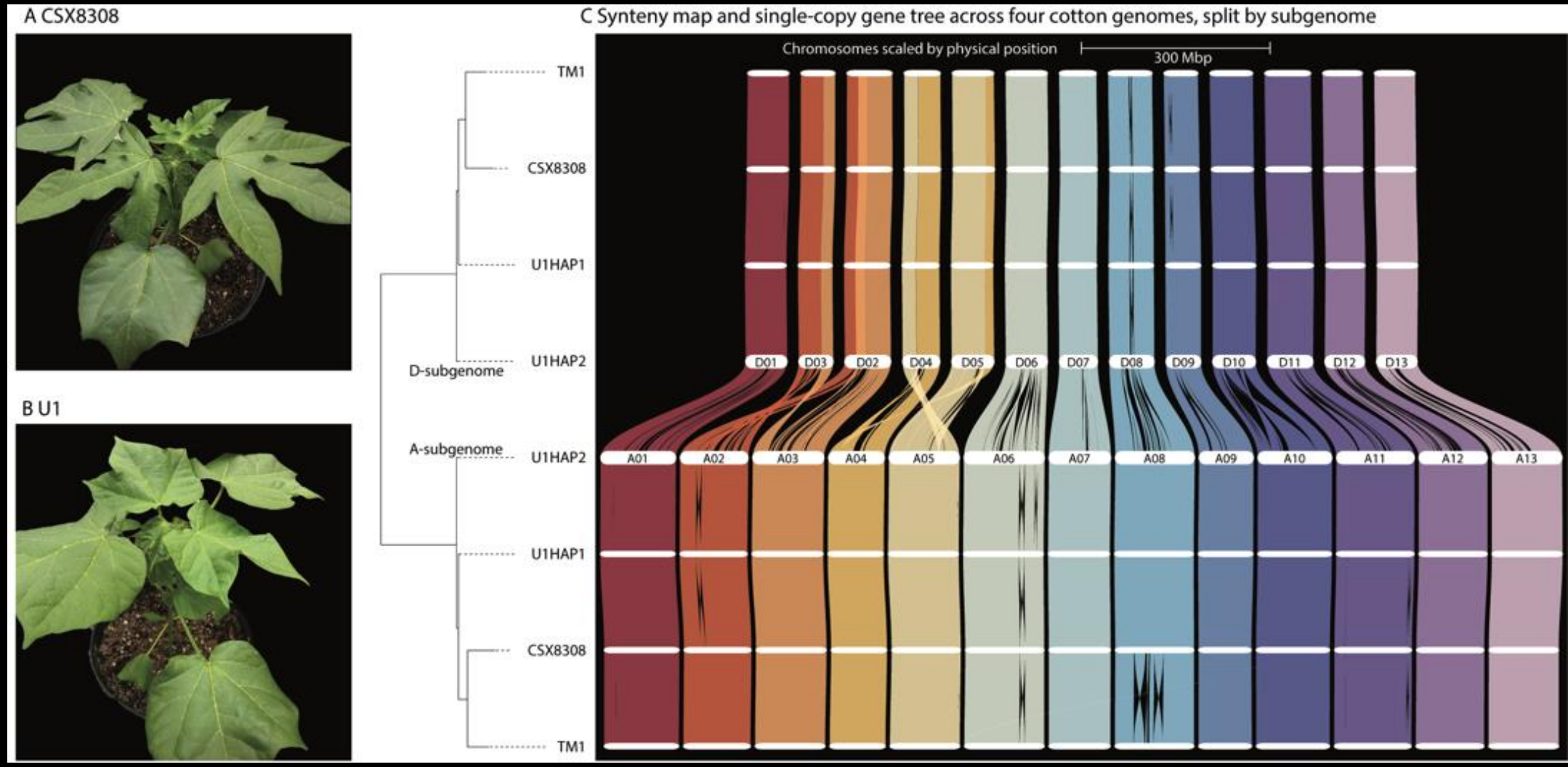
Gene discovery

Gupta, M., Praveen, A. (2019). Quantitative Trait Loci Mapping of Heavy Metal Accumulation and Resistance in Crop Plants.

Unique genomic resources

FOV4 Susceptible

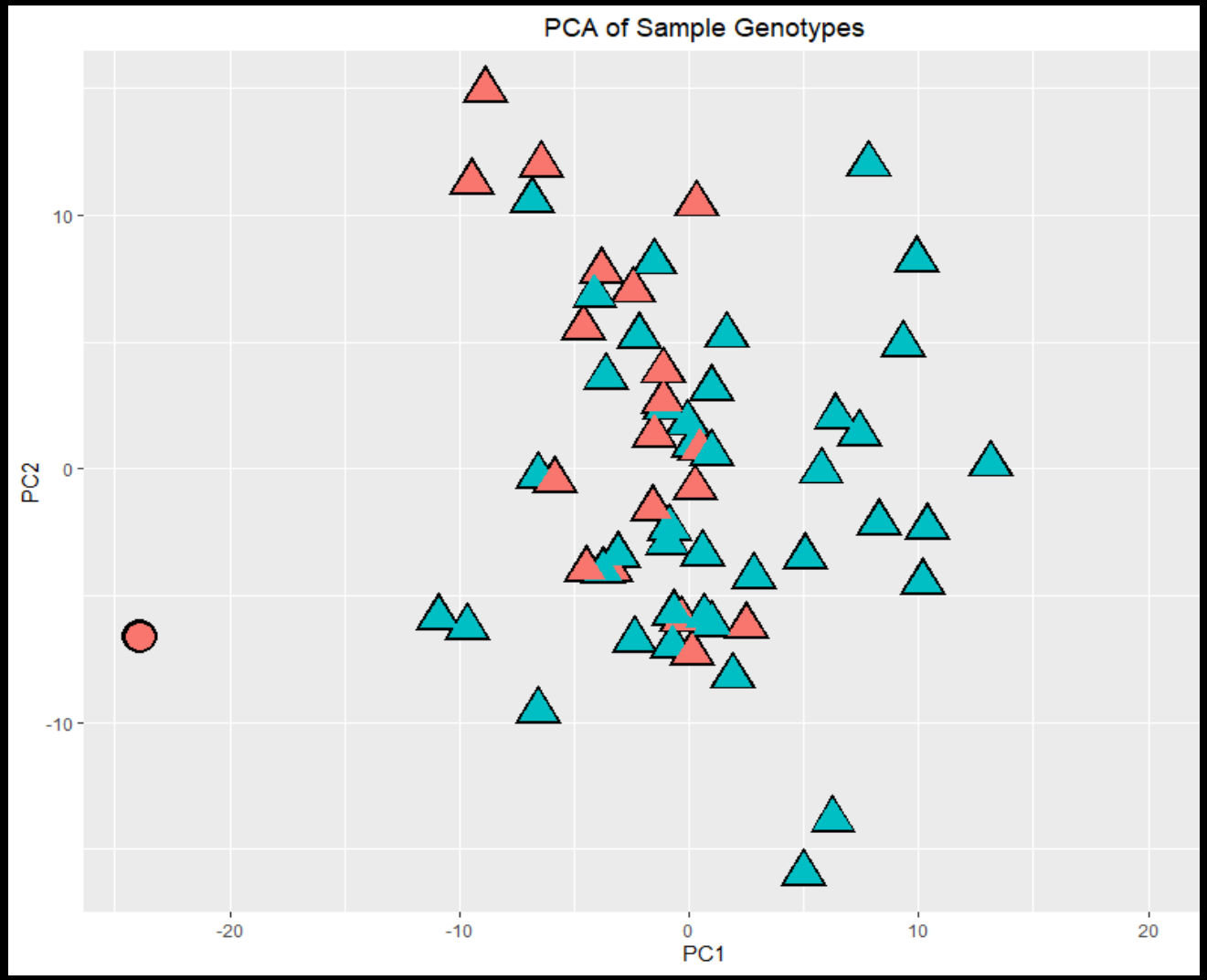
FOV4 Resistant



Parris et al. (2024) *In Review*

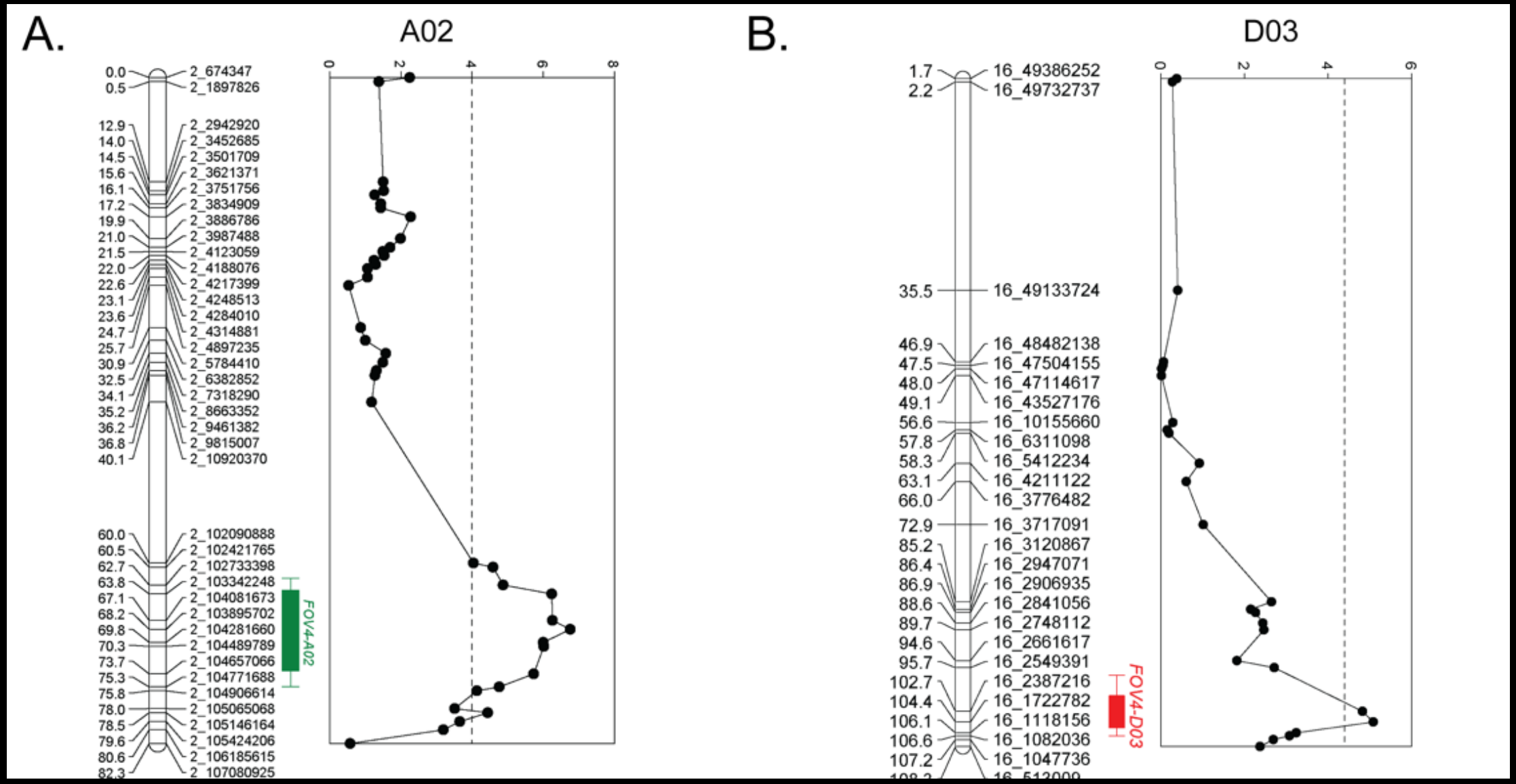
Skim sequencing F3 segregants

- 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

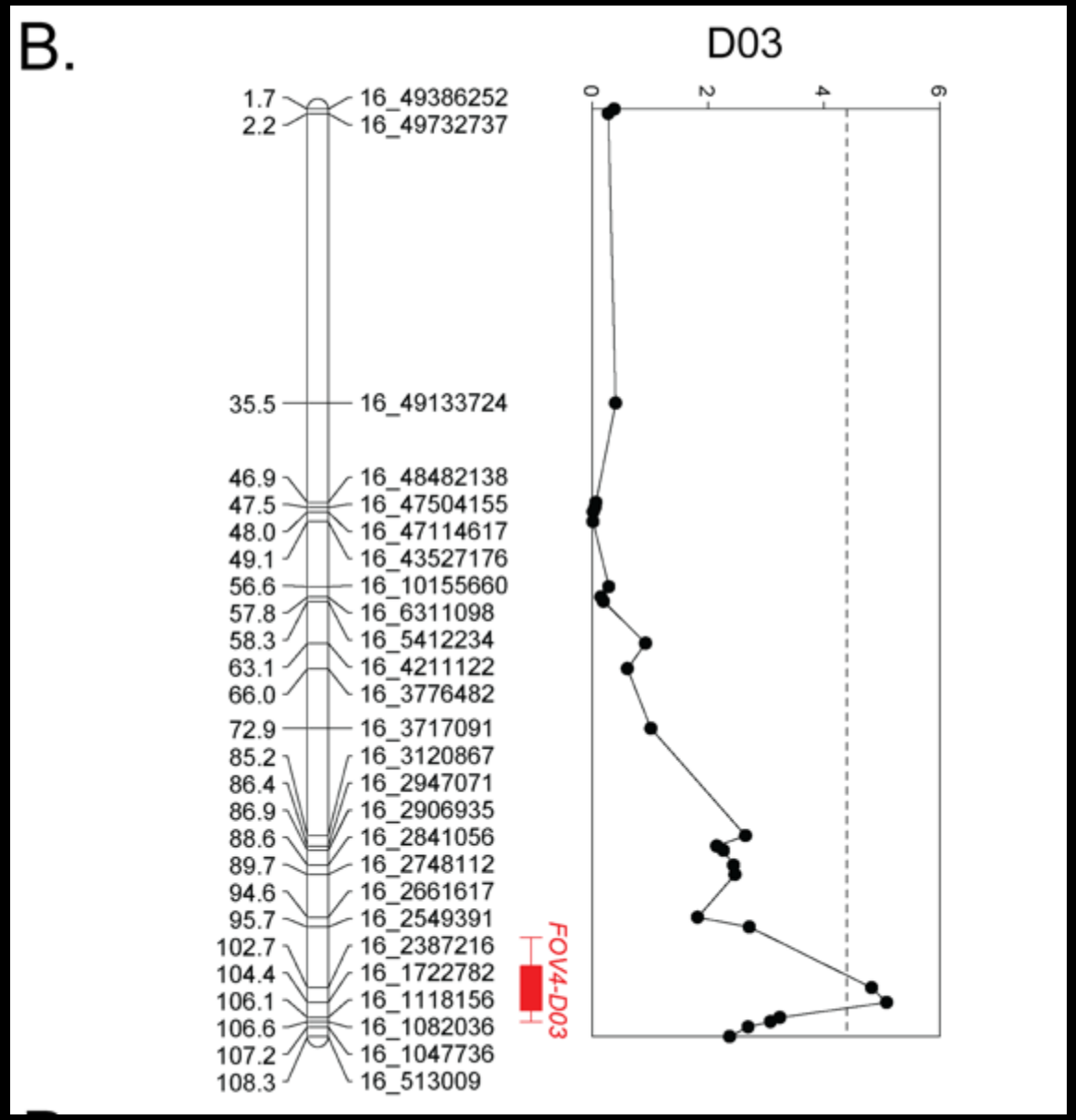


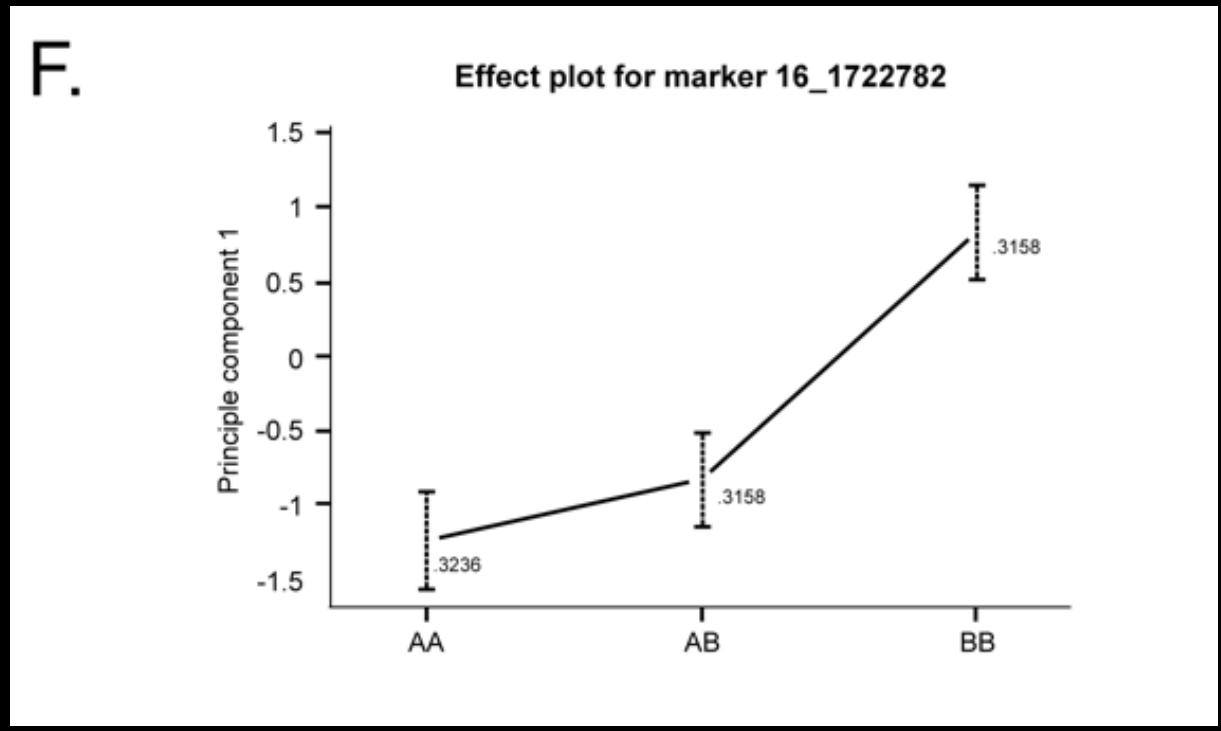
Parris et al. (2024) *In Review*

Two major QTLs significantly associated with FOV4 resistance



Parris et al. (2024) *In Review*





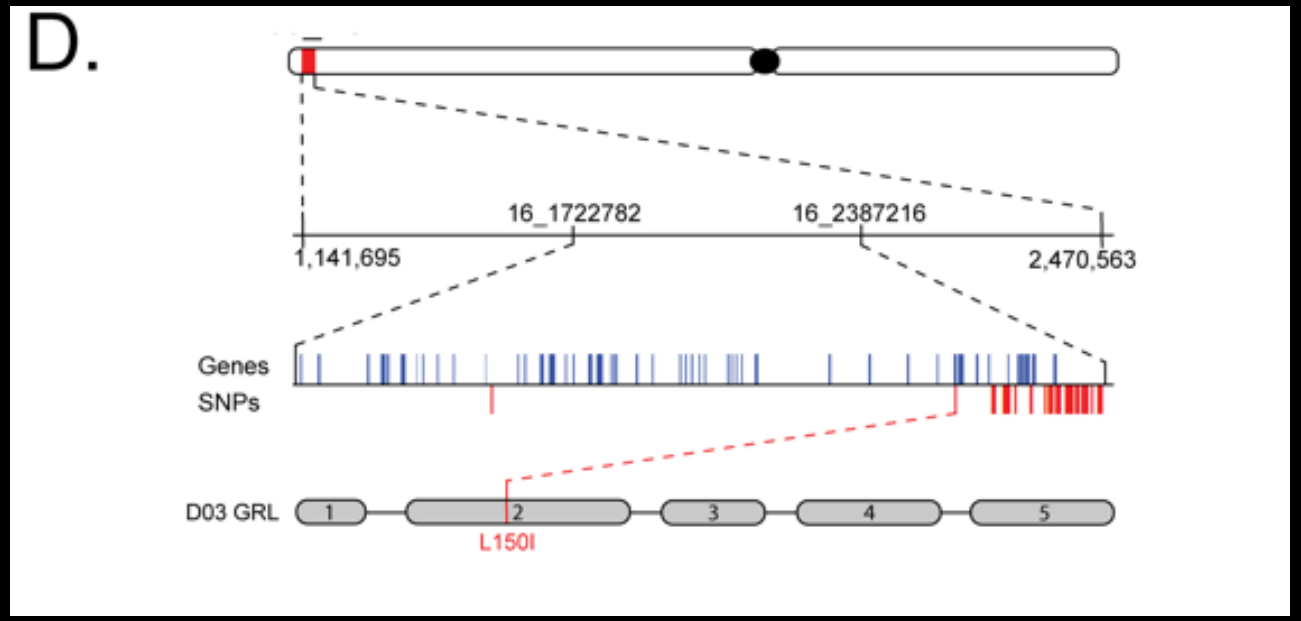
QTL on chromosome D03

1.3 Megabases
102 genes

Parris et al. (2024) *In Review*

Parris et al. (2023) *In Review. PNAS*

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		--									
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		--									
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

L150I

Susceptible

A R E I A S L I G D Y L W L K P G T T V Y

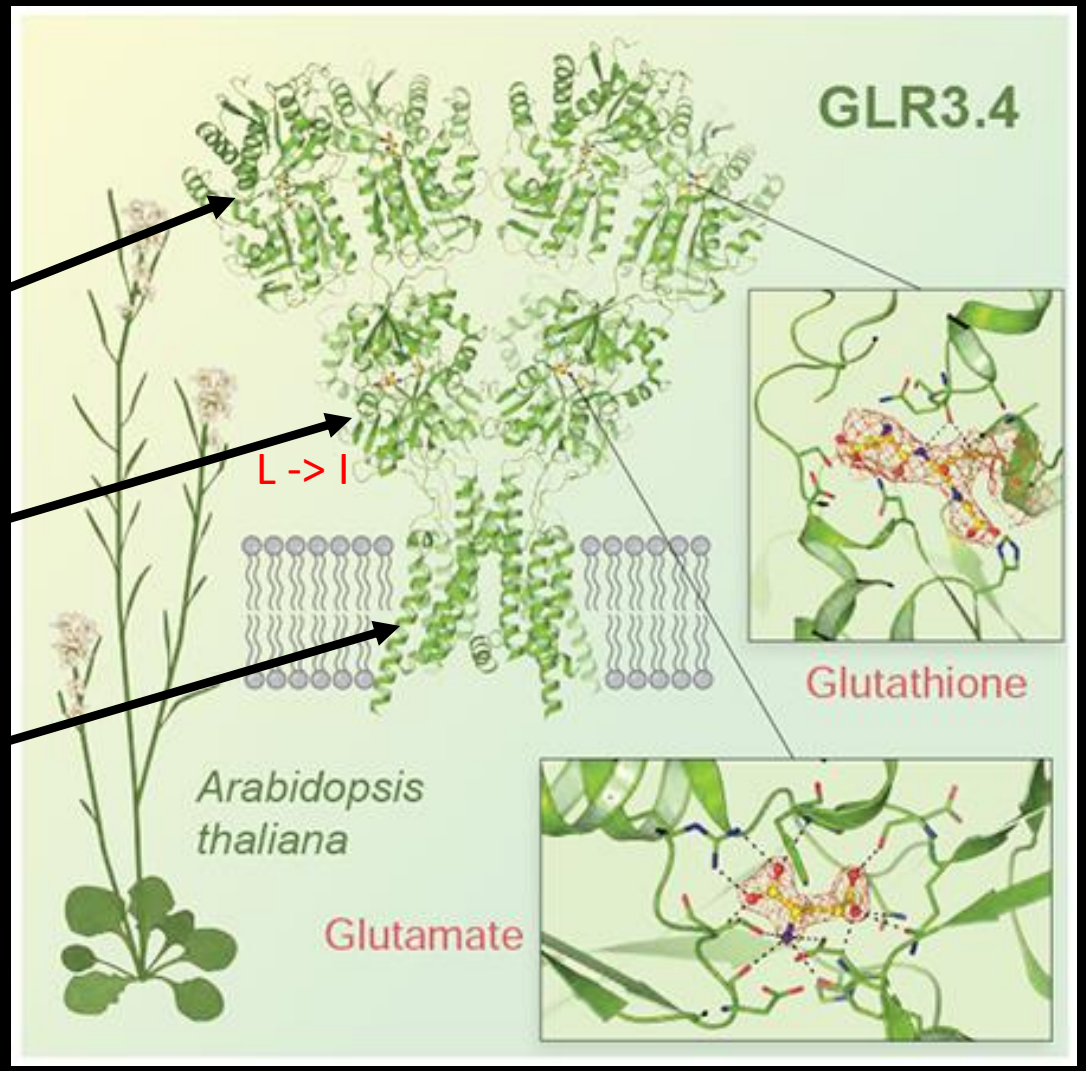
A R E I A S L I G D Y I W L K P G T T V Y

Resistant

Amino terminal

Ligand binding

Transmembrane*



Mol Cell. 2021 Aug 5; 81(15): 3216–3226.e8.

ADVANCED SCIENCE Open Access

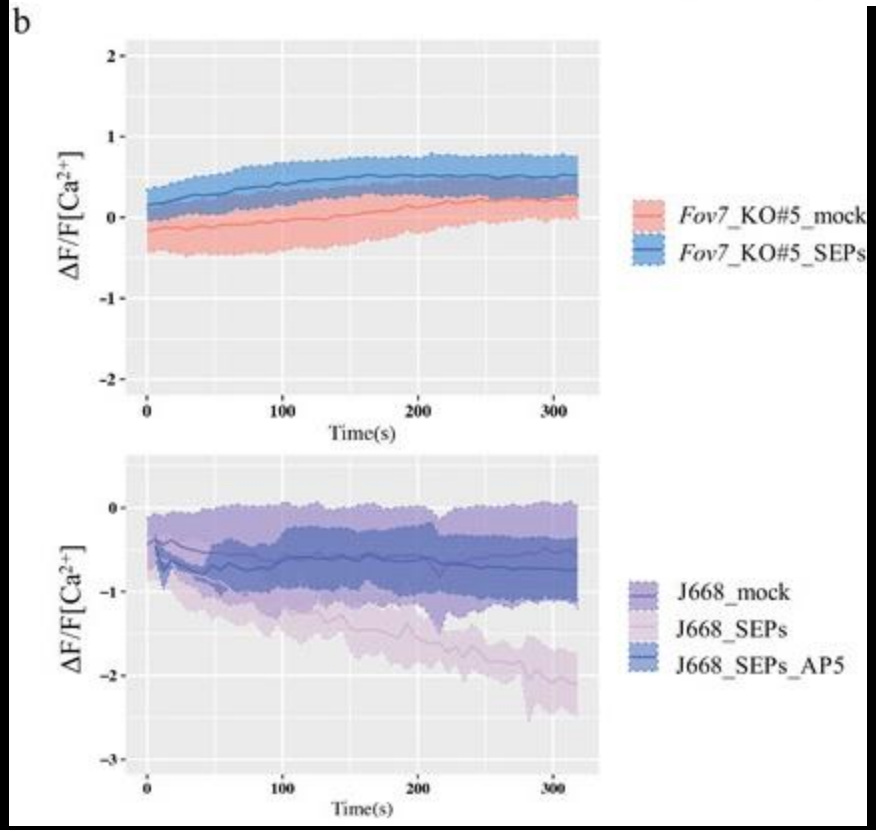
Adv Sci (Weinh). 2021 Apr; 8(7): 2002723. PMCID: PMC8025038
Published online 2021 Feb 19. doi: 10.1002/advs.202002723 PMID: 33854882

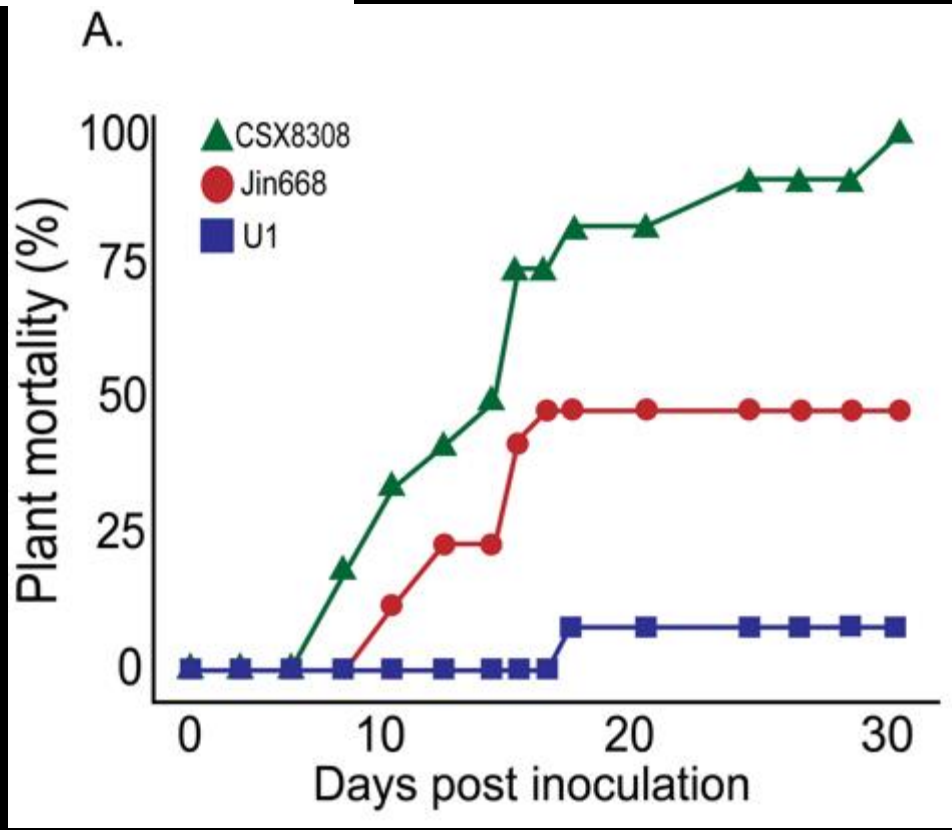
A Single-Nucleotide Mutation in a GLUTAMATE RECEPTOR-LIKE Gene Confers Resistance to Fusarium Wilt in *Gossypium hirsutum*

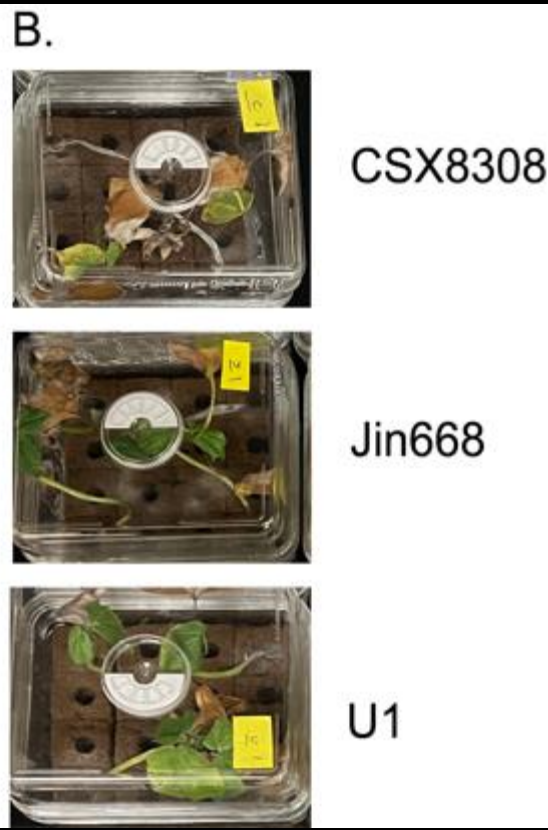
Shiming Liu,[1] Xiaojun Zhang,[1] Shenghua Xiao,[1] Jun Ma,[2] Weijun Shi,[2] Tao Qin,[1] Hui Xi,[3] Xinhui Nie,[3] Chunyuan You,[4] Zheng Xu,[1] Tianyi Wang,[1] Yujing Wang,[1] Zhennan Zhang,[1] Jianying Li,[1] Jie Kong,[2] Alifu Aierxi,[2] Yu Yu,[5] Keith Lindsey,[6] Steven J. Klosterman,[7] Xianlong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

FOV7 in China

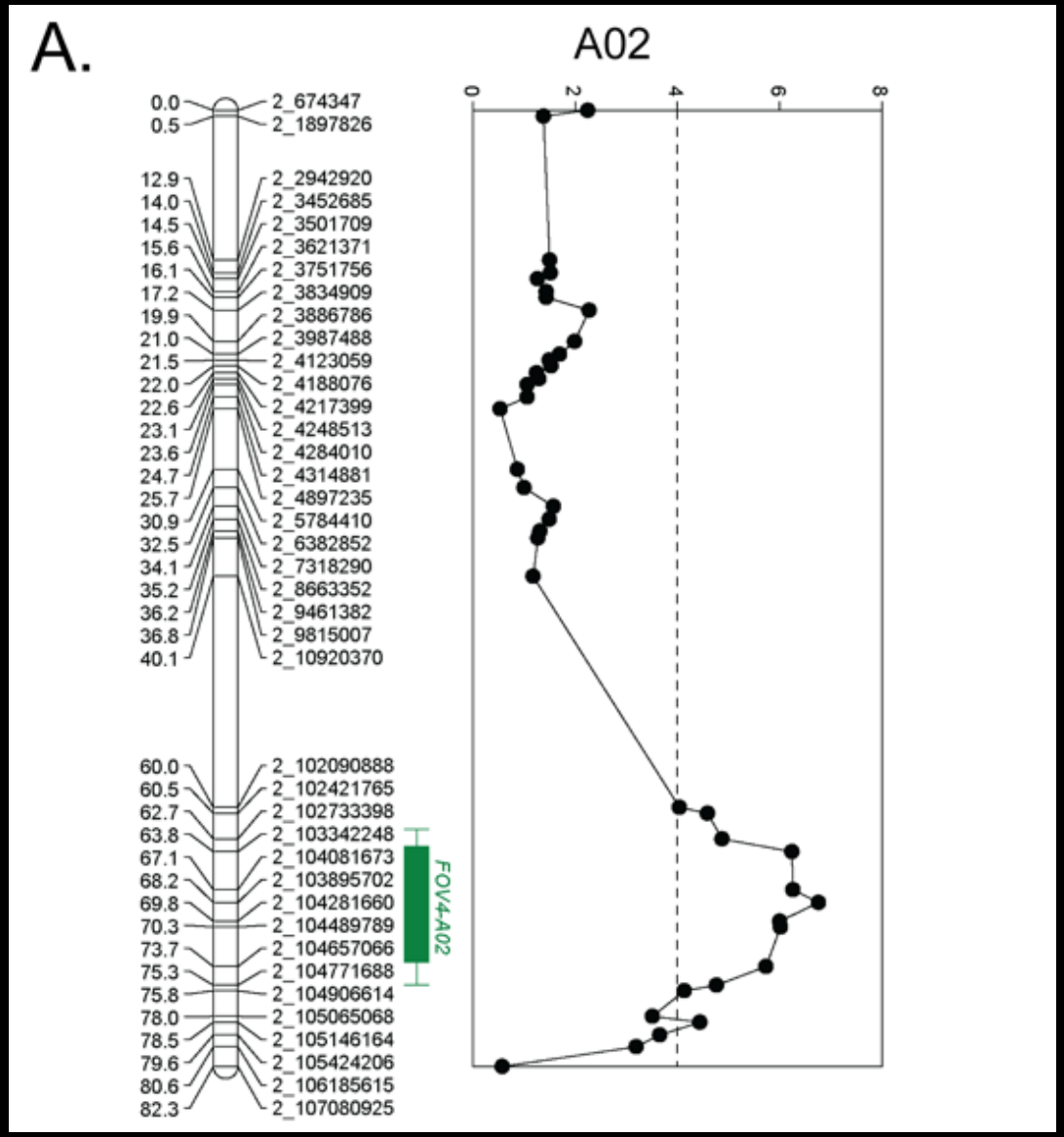
D03 GRL contribution to FOV7 and FOV4 resistance and cytosolic calcium influx

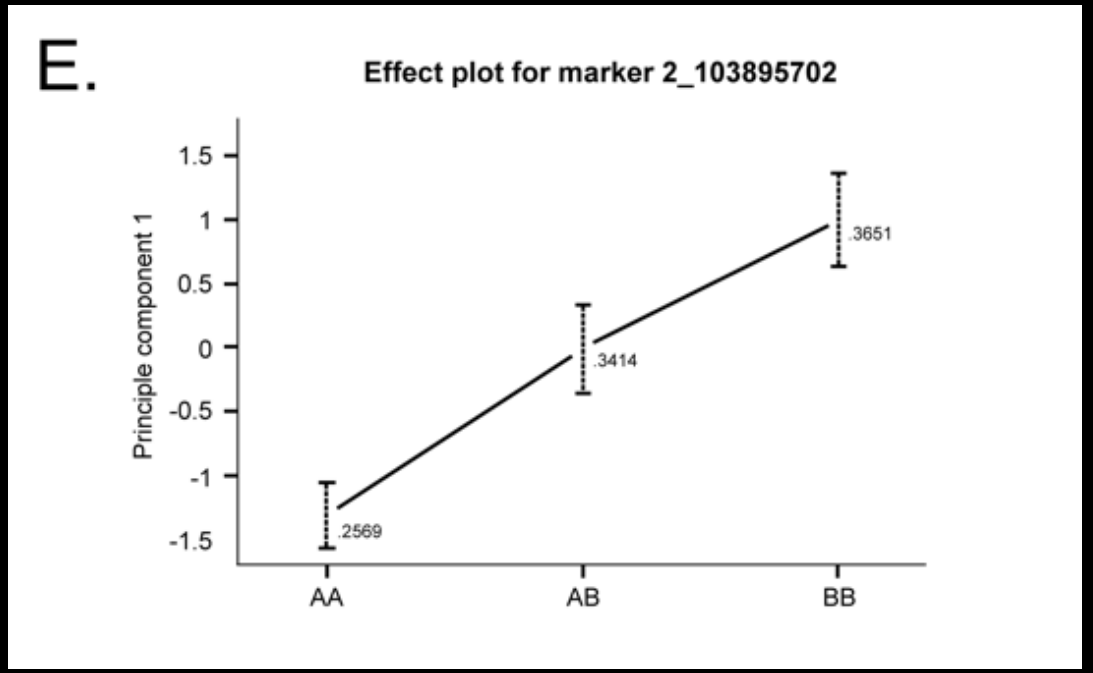






Parris et al. (2024) *In Review*





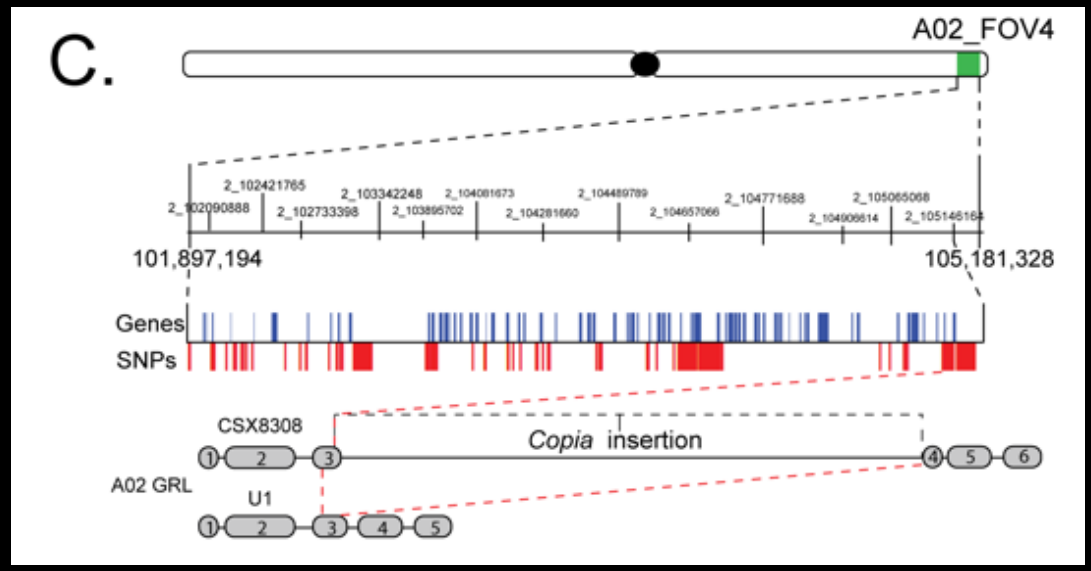
QTL on chromosome A02

3.3 Megabases
163 genes

Parris et al. (2024) *In Review*

A02 QTL gene discovery

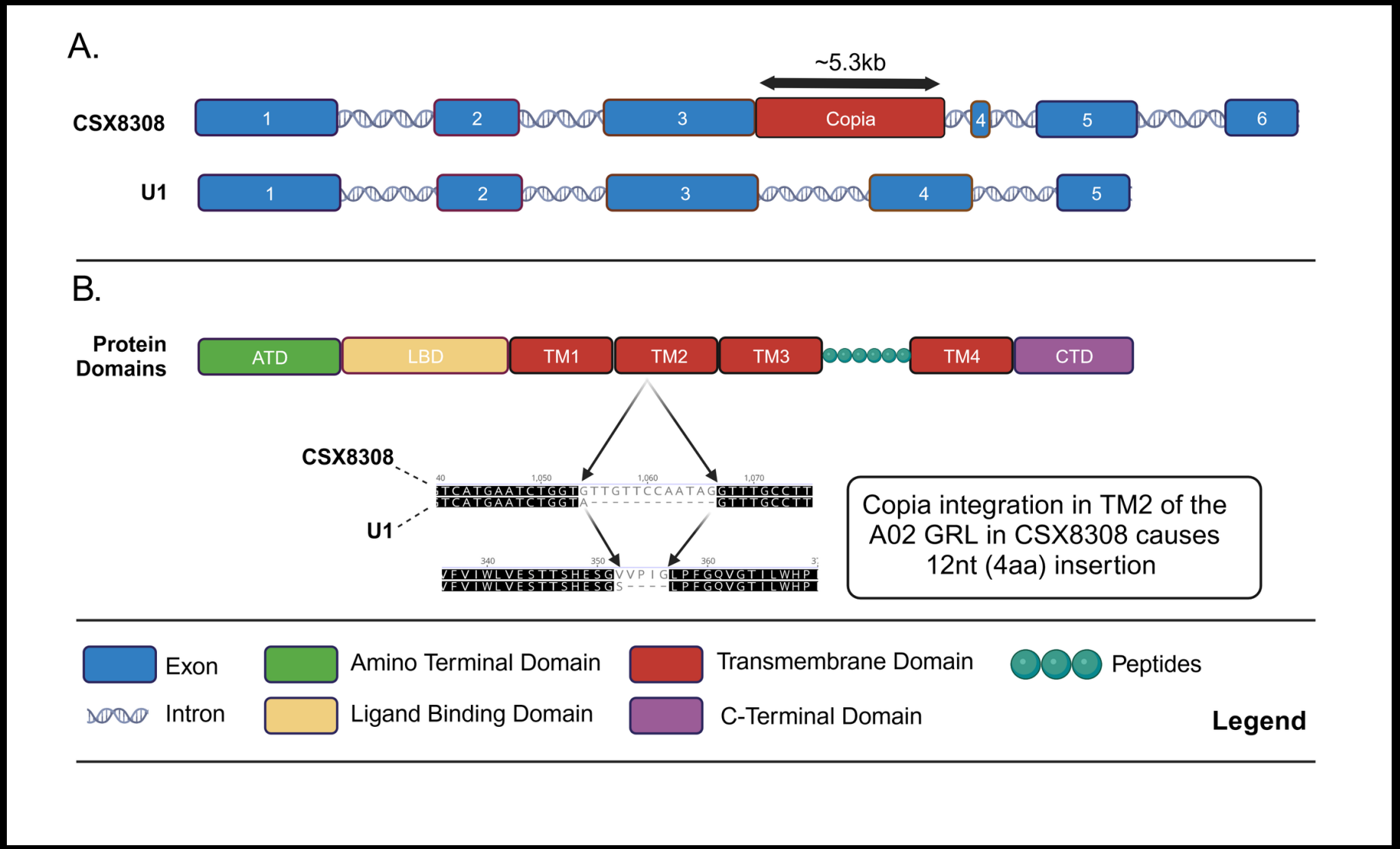
Parris et al. (2023) *In Review. PNAS*



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	#N/A	BB	AA	AB	AA	BB	AB	AA	AA	AA	AA	AA
A02	103830935	G	T	GhCSX8308.A02G180300	#N/A	BB	AA	AB	AA	BB	AB	AA	AA	AA	AA	AA
A02	103832224	T	G	GhCSX8308.A02G180300	#N/A	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	#N/A	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



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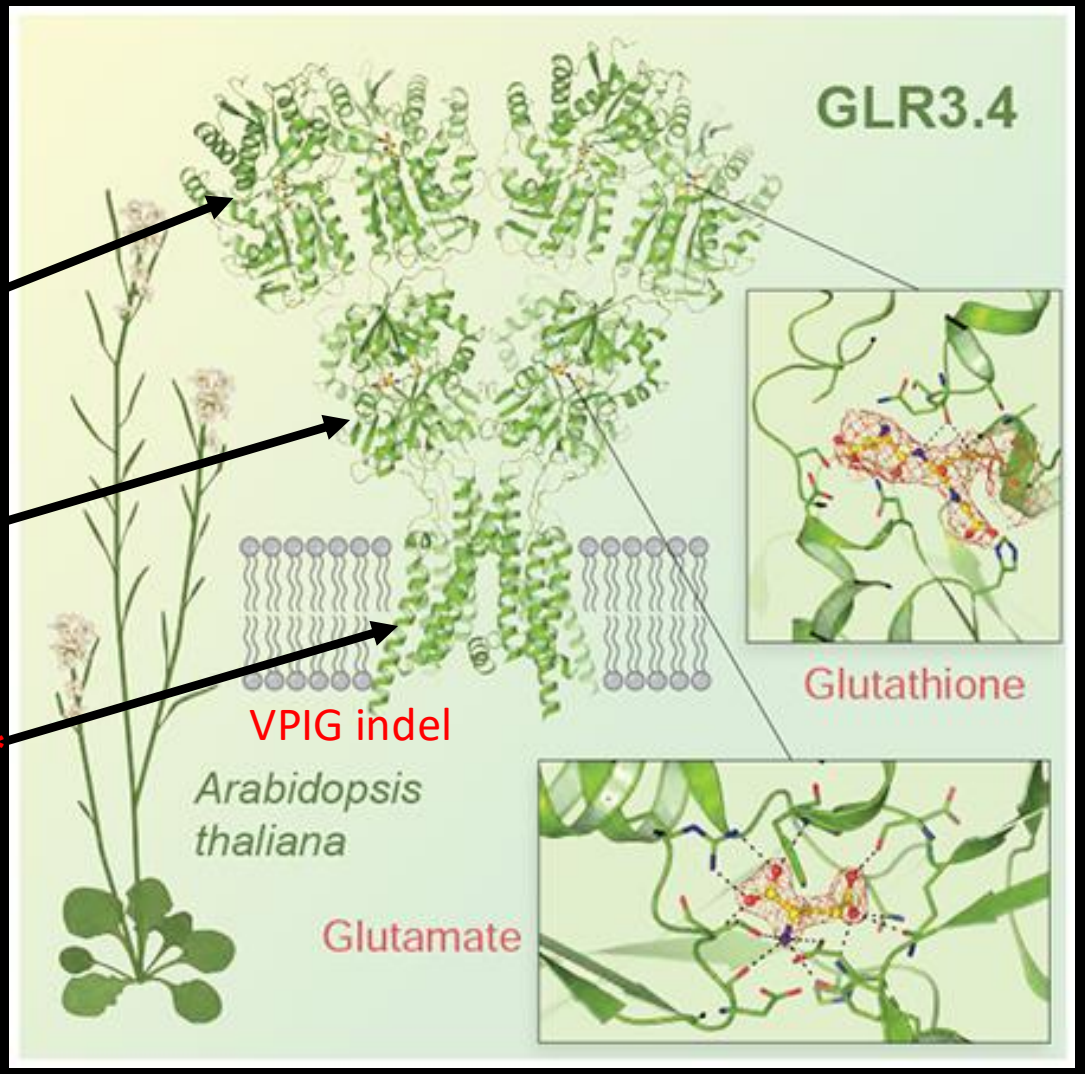
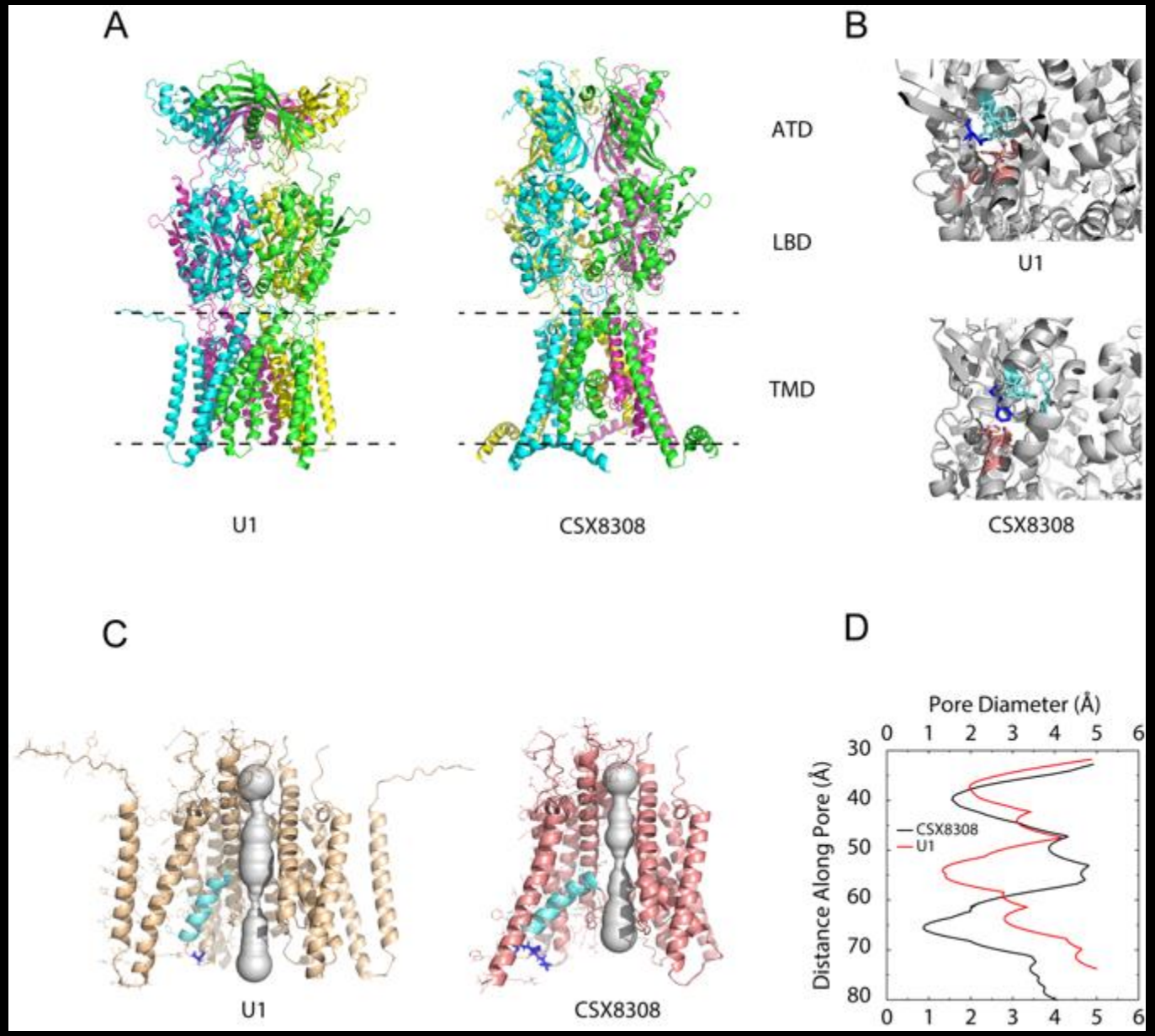
Plant glutamate receptors



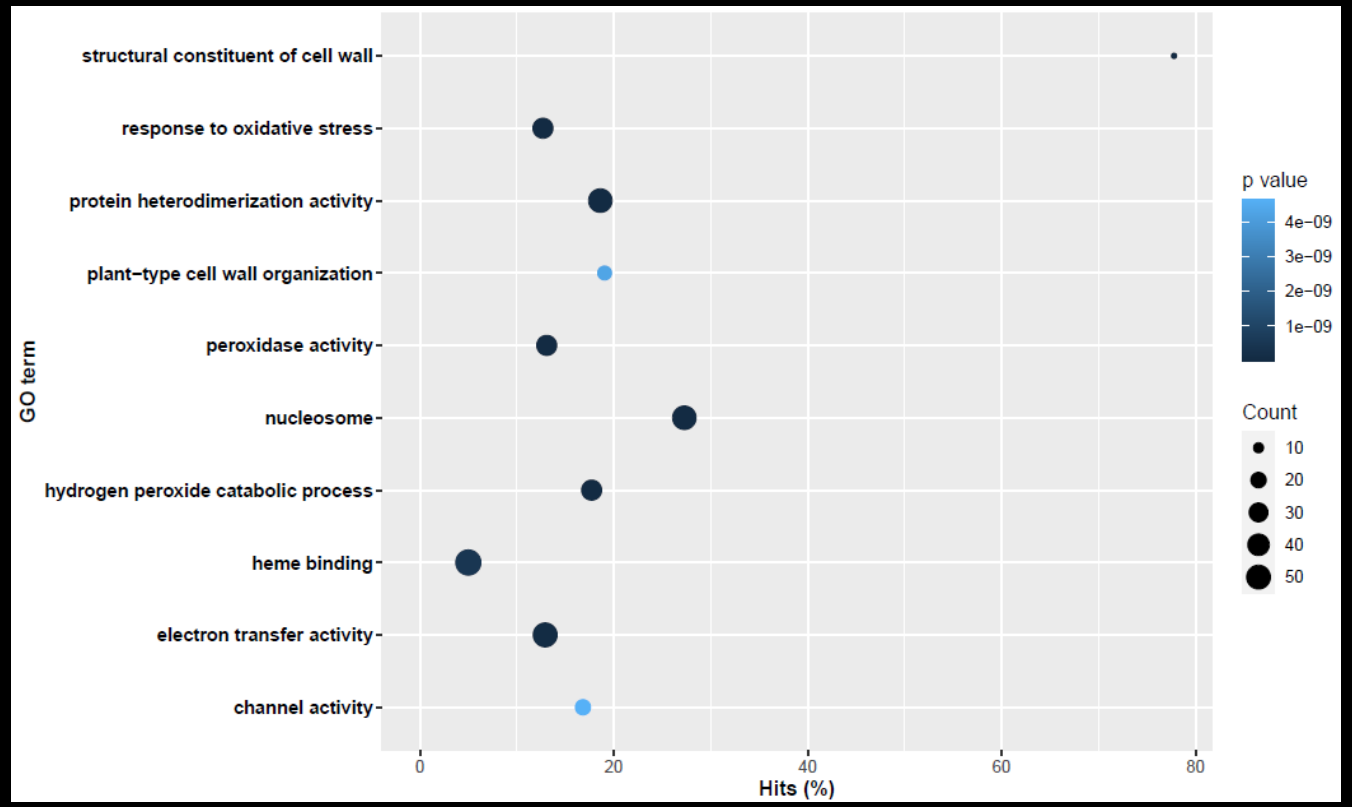
Mol Cell. 2021 Aug 5; 81(15): 3216–3226.e8.

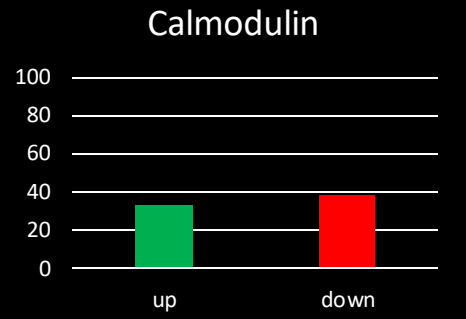
A02 GLR pore size in U1 is larger

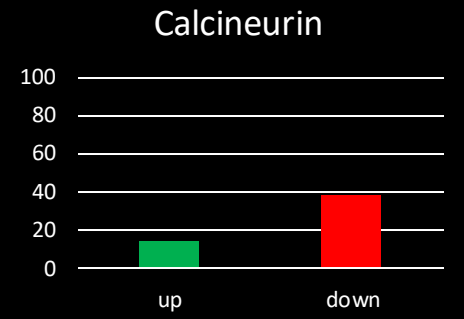


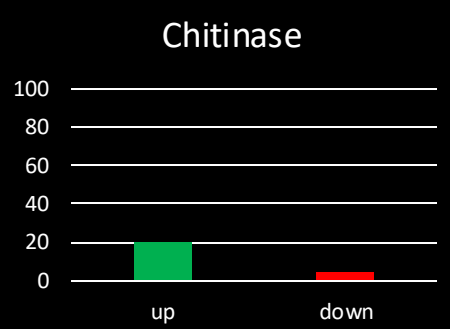
Parris et al. (2024) *In Review*

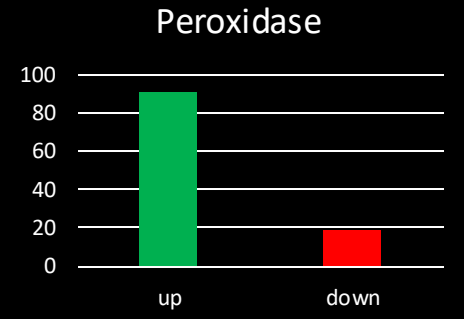
Transcriptomics underlying FOV4 resistance in ‘U1’

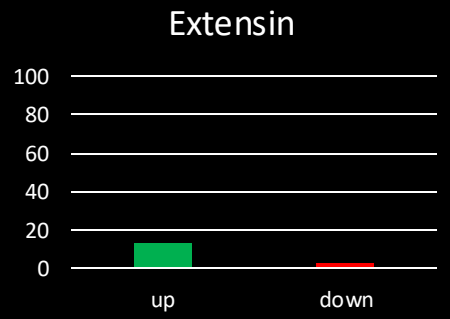


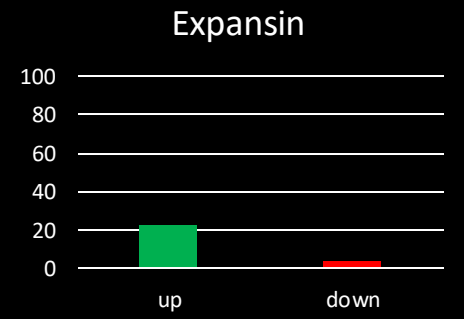




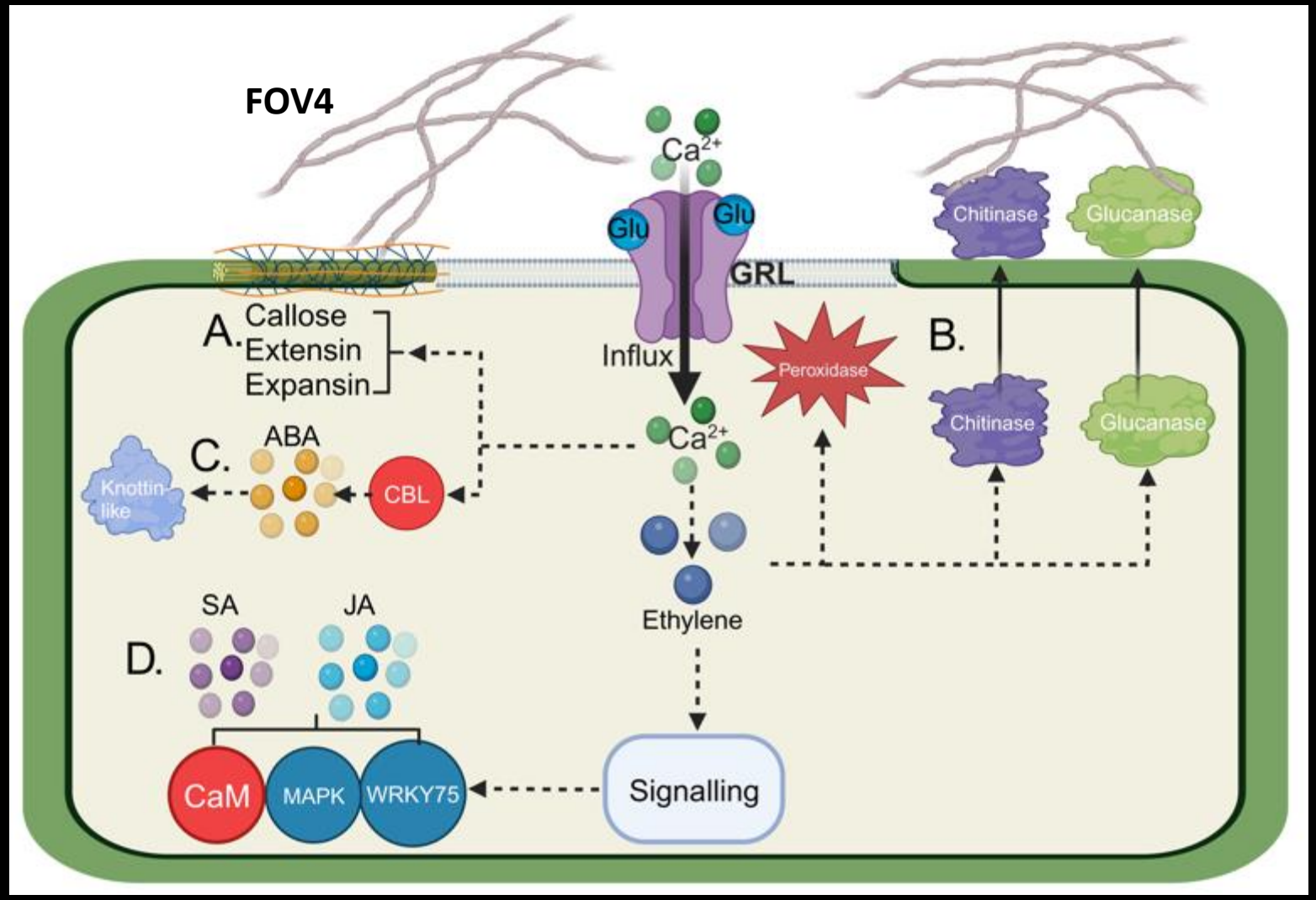








Potential GRL-mediated mechanisms for FOV4 resistance

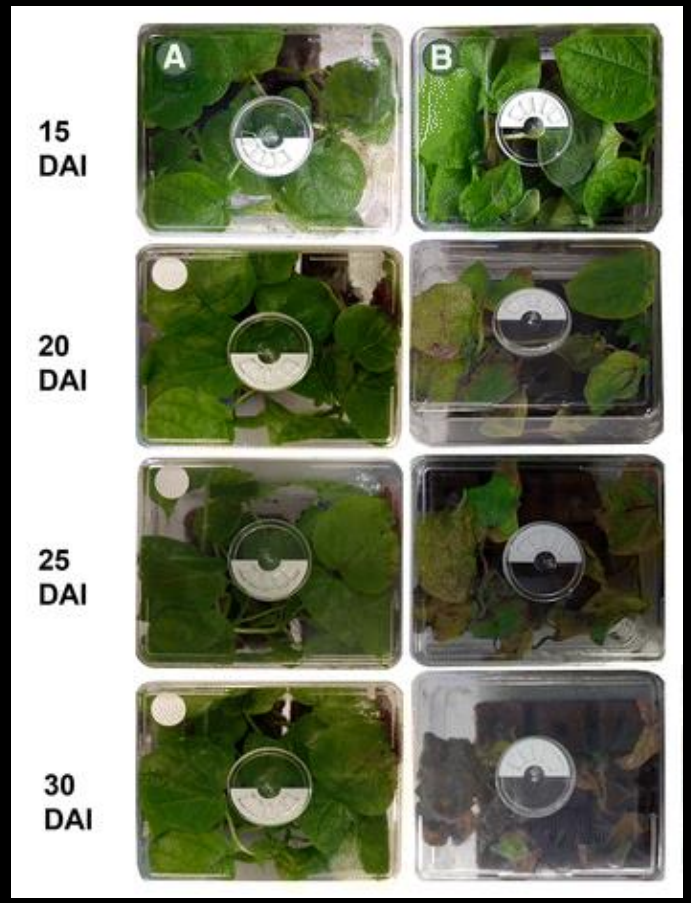


Parris et al. (2024) *In Review*

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

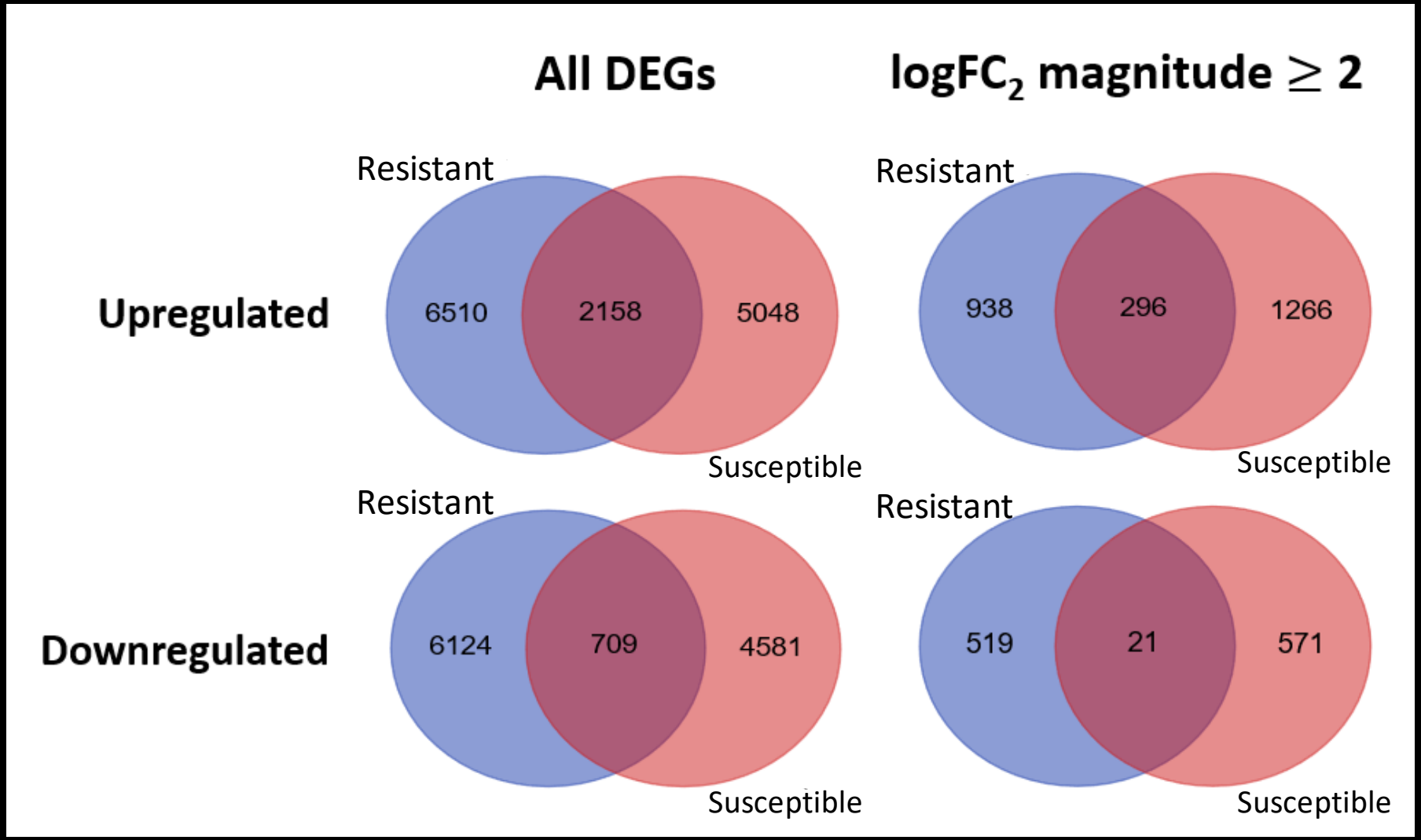


Parris et al. (2023) *Plant Disease*

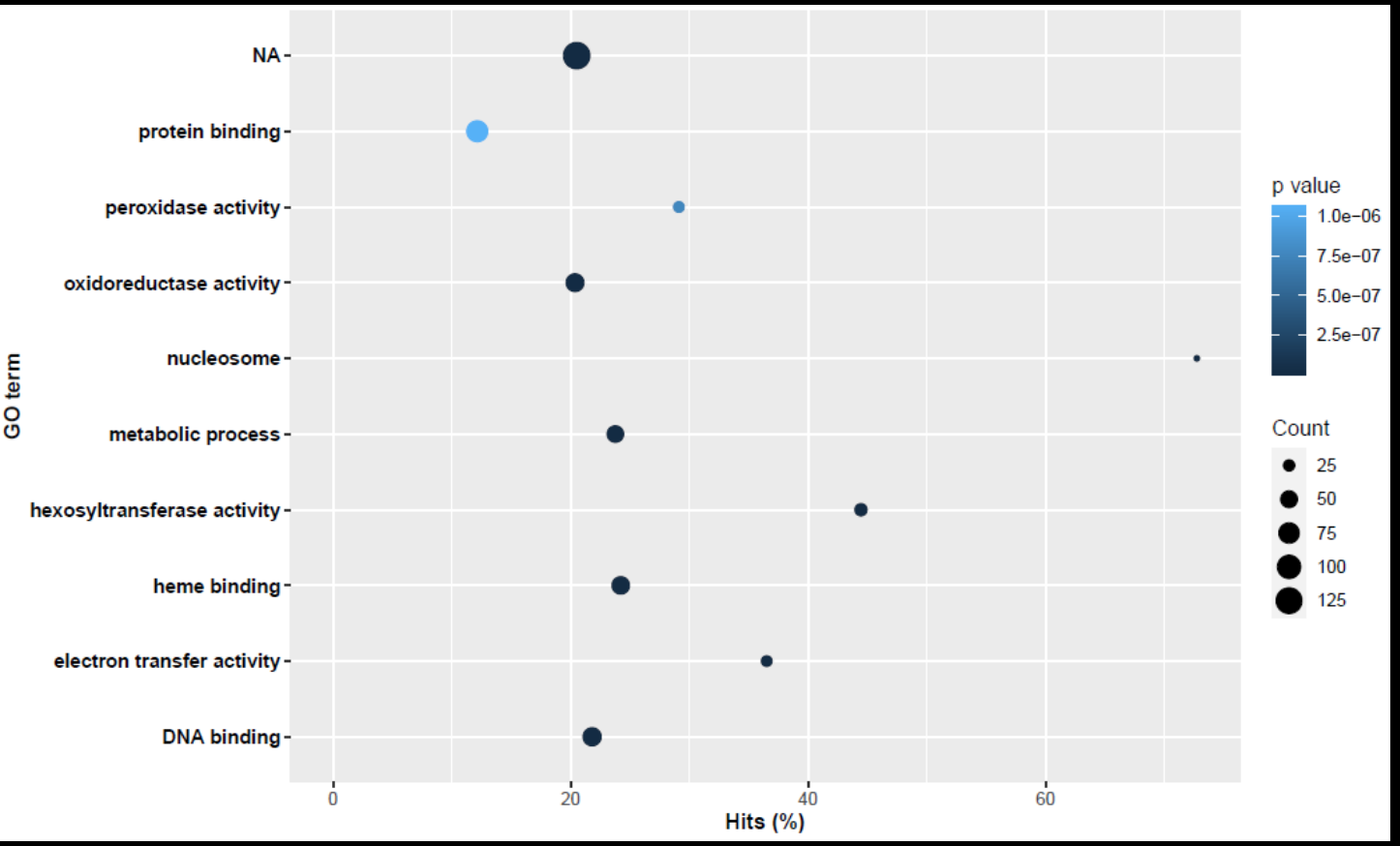
Collected tissue at 3 time points:

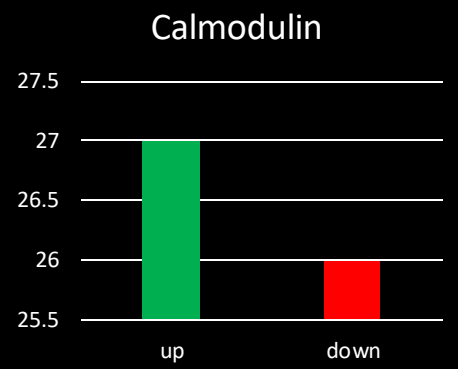
- 12hai, 24hai, 72hai
- Extract RNA for RNAseq
- Identify unique expression in resistant pima

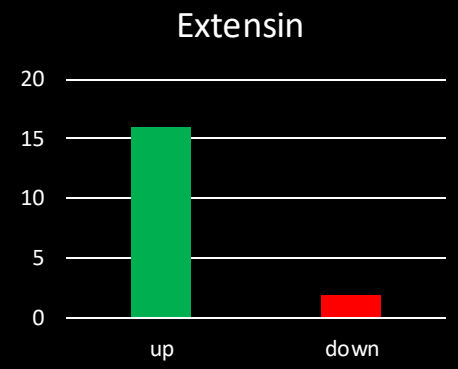
Induced gene counts

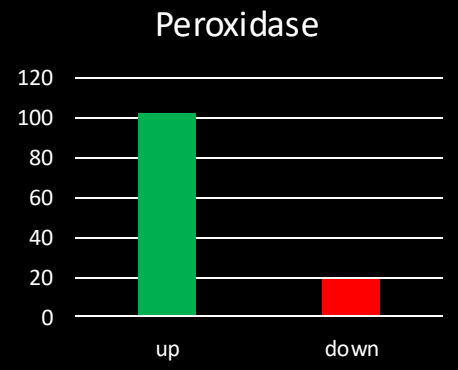


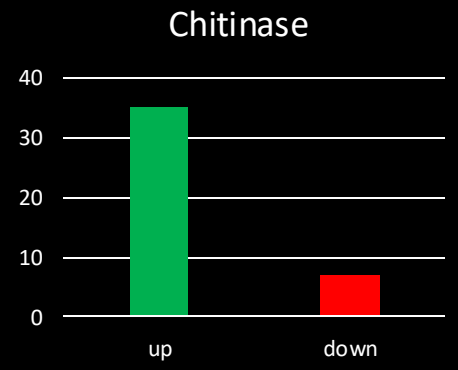
GO enrichment



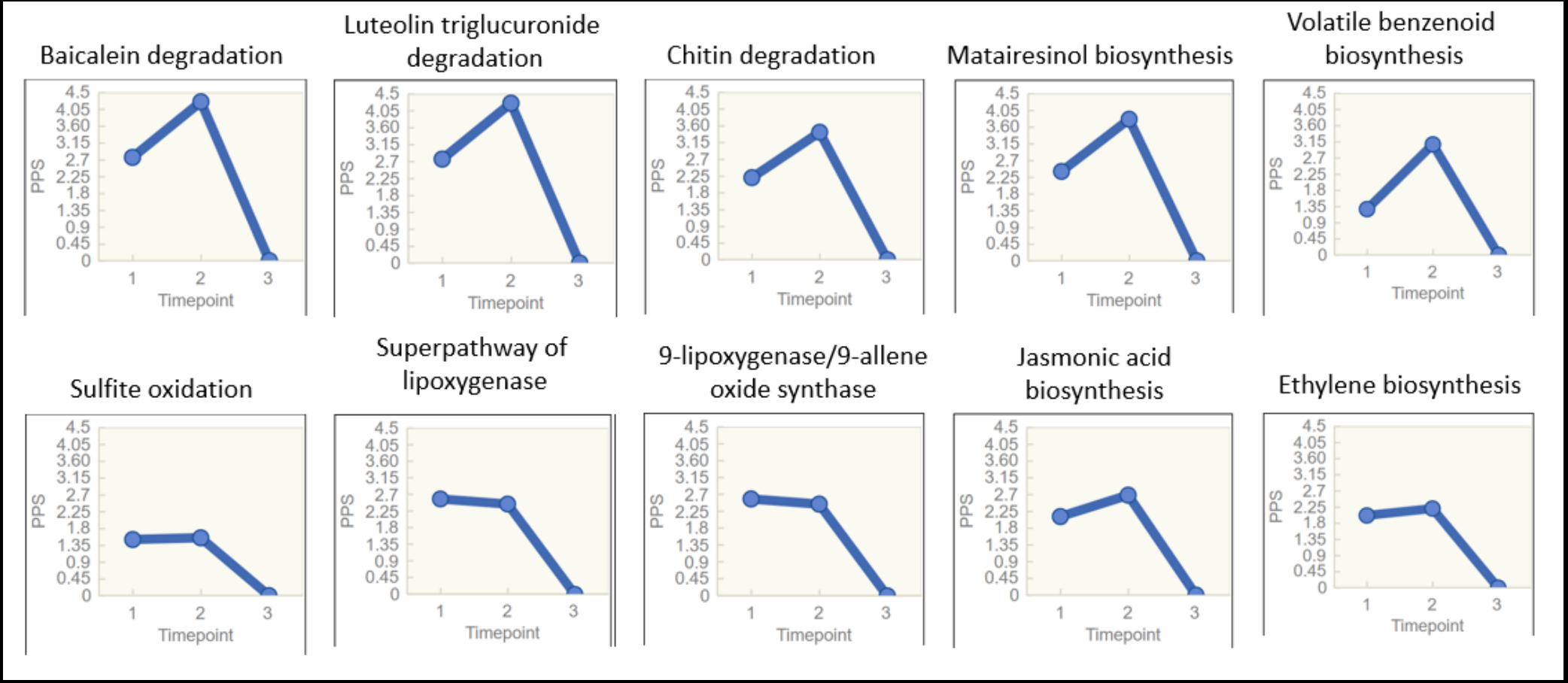


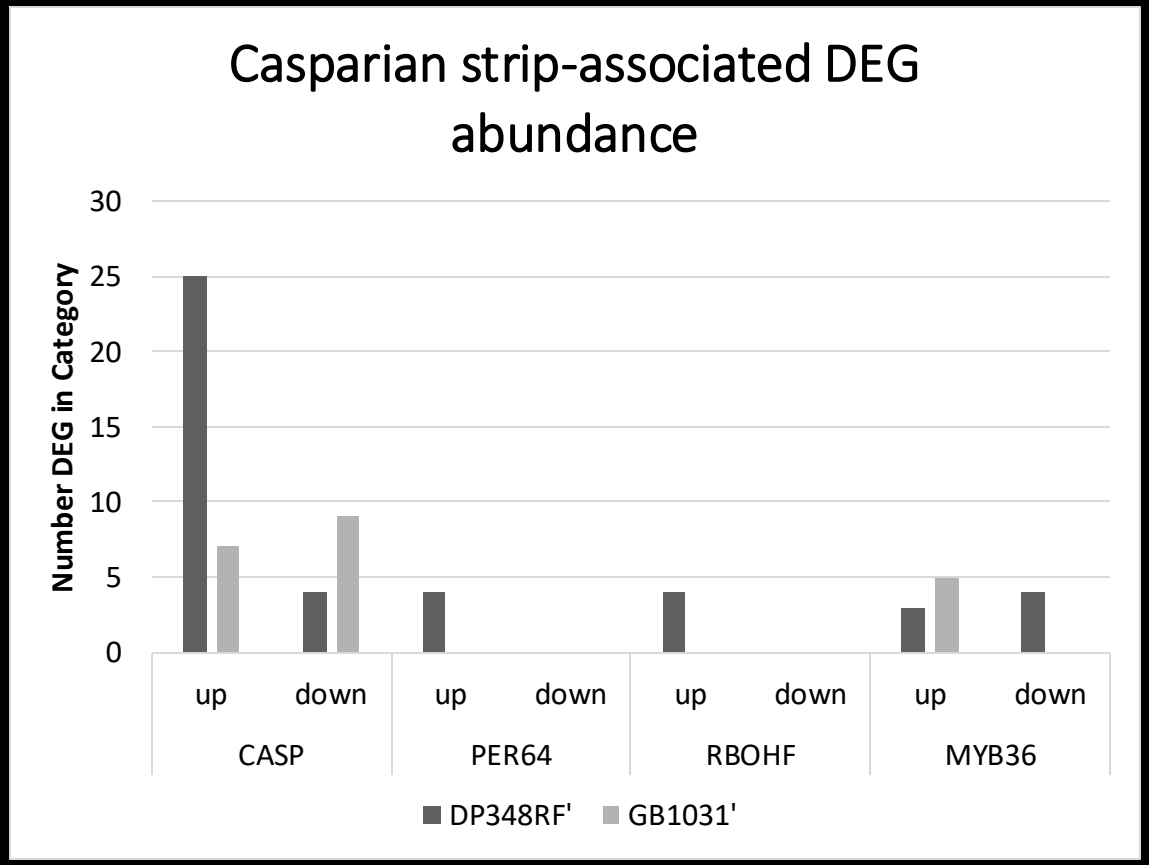


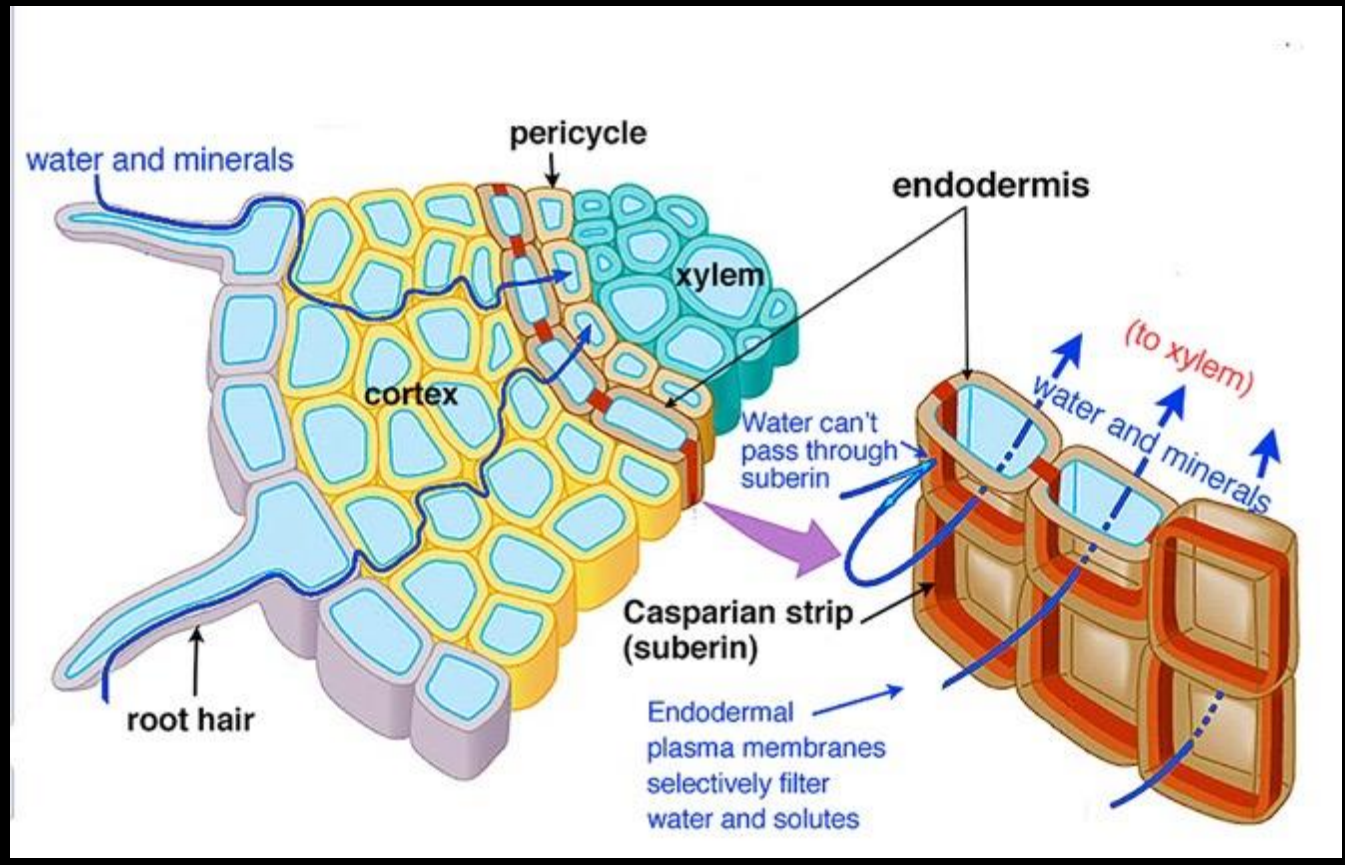




Biochemical pathway enrichment

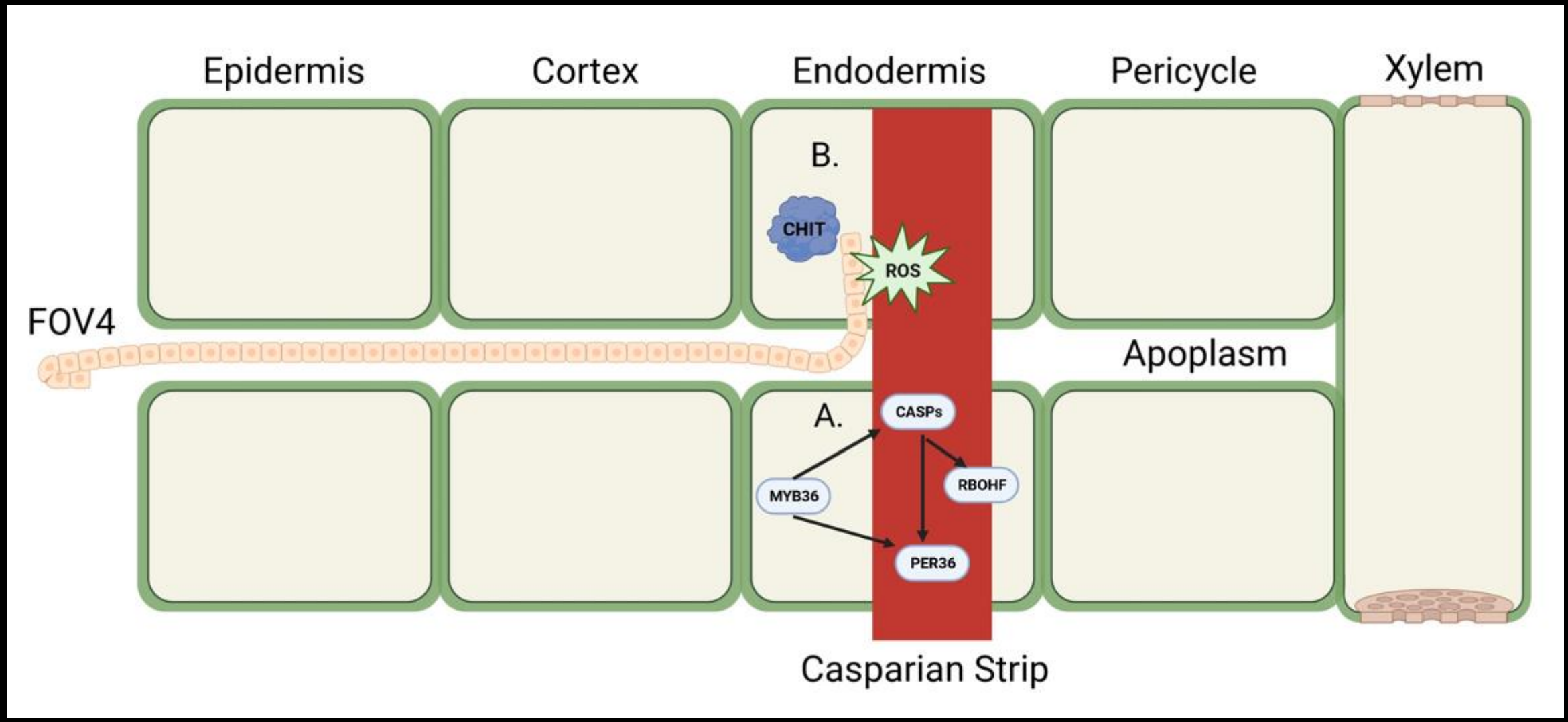






https://www.bio.miami.edu/dana/pix/endodermis_cells.jpg

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



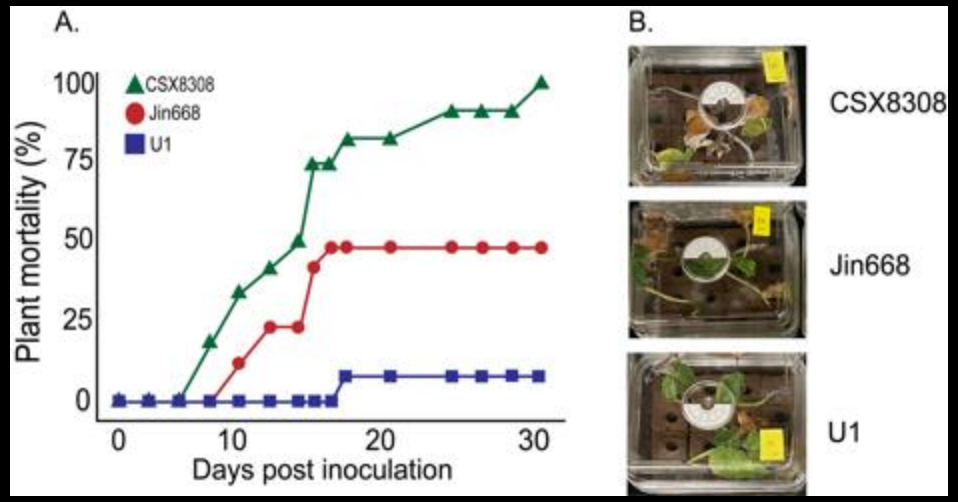
Conclusions

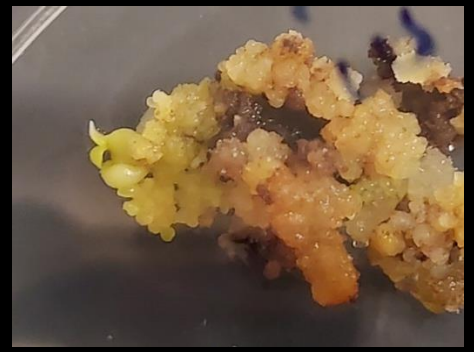
- Reactive oxidative bursts underly FOV4 resistance in both species
- Wall off mechanisms play pivotal role in FOV4 resistance in both upland and pima
  - General cell wall fortification
  - Casparian strip fortification provides a biochemical barrier against vascular pathogen invasion
- GLRs are new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

Ongoing Work

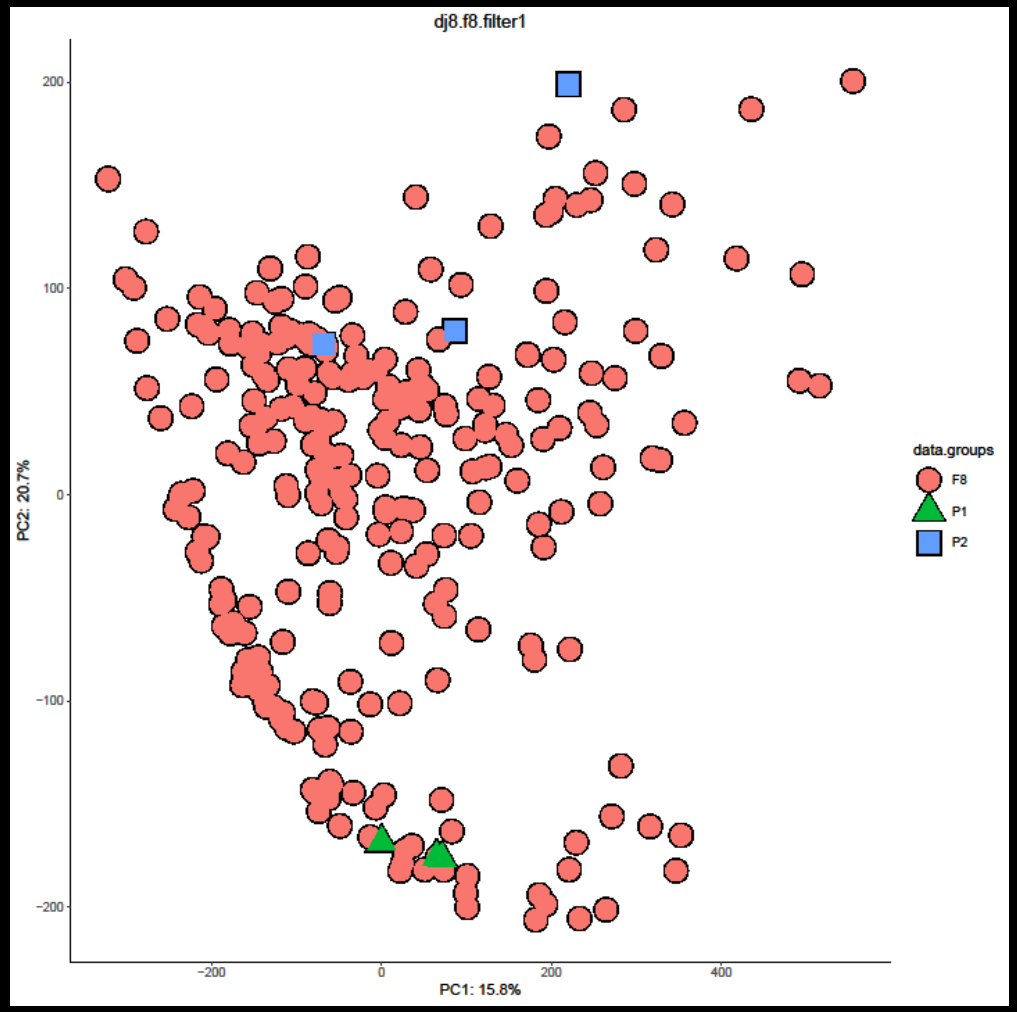
Gene Validation

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)



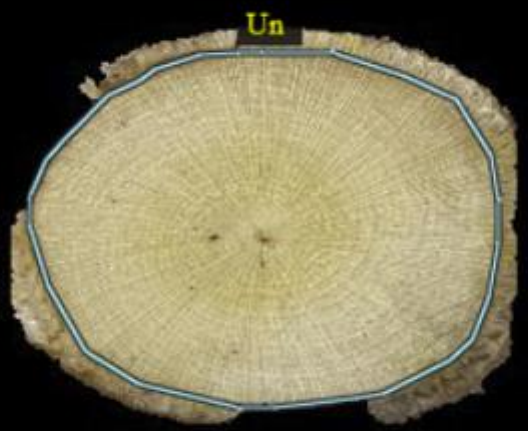
- F8 RIL population (U1 x CSX8308)
- Phenotyped in El Paso, Fall 2023
- Skim sequenced 3-5X (HA)
- >5k quality SNPS

Cotton Cross Section Stain Analysis - Computer Vision

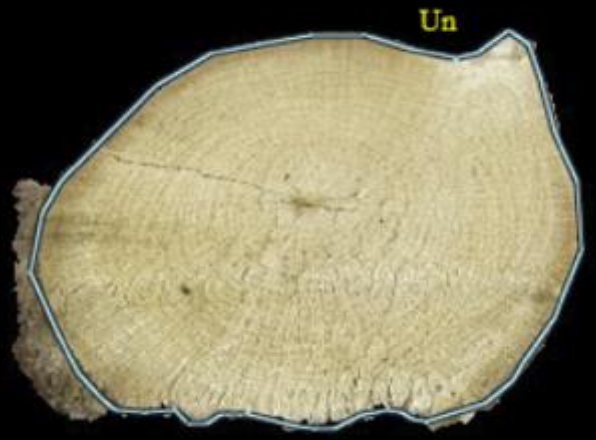
DJ-8-110

Total vascular tissue area: 13351.5 (pixels)
Total stained area: 0
Percent area stained: 0%

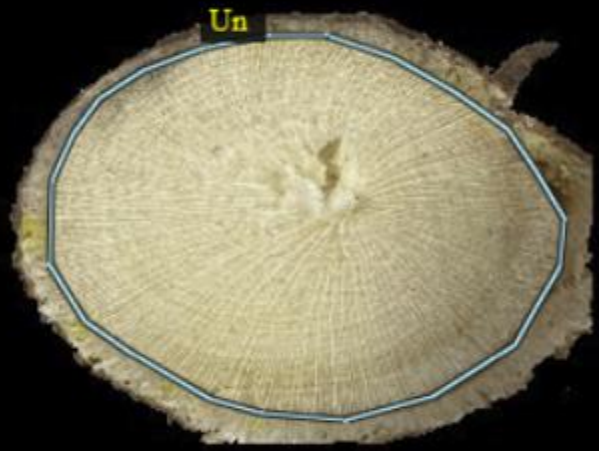
0% Stain Examples



DJ-8-165



DJ-8-113

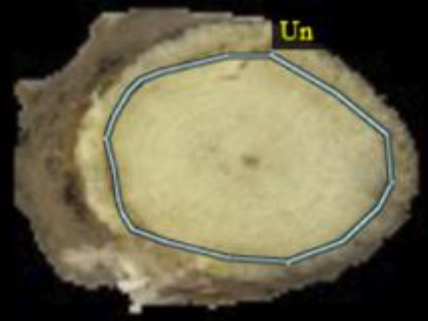


DJ-8-115

Total Surface Area Variability

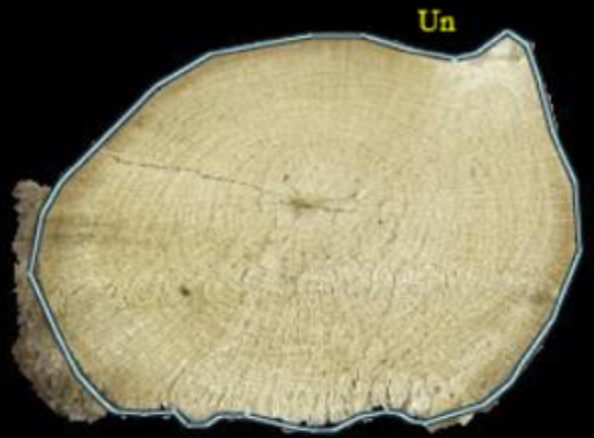
DJ-8-77

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%

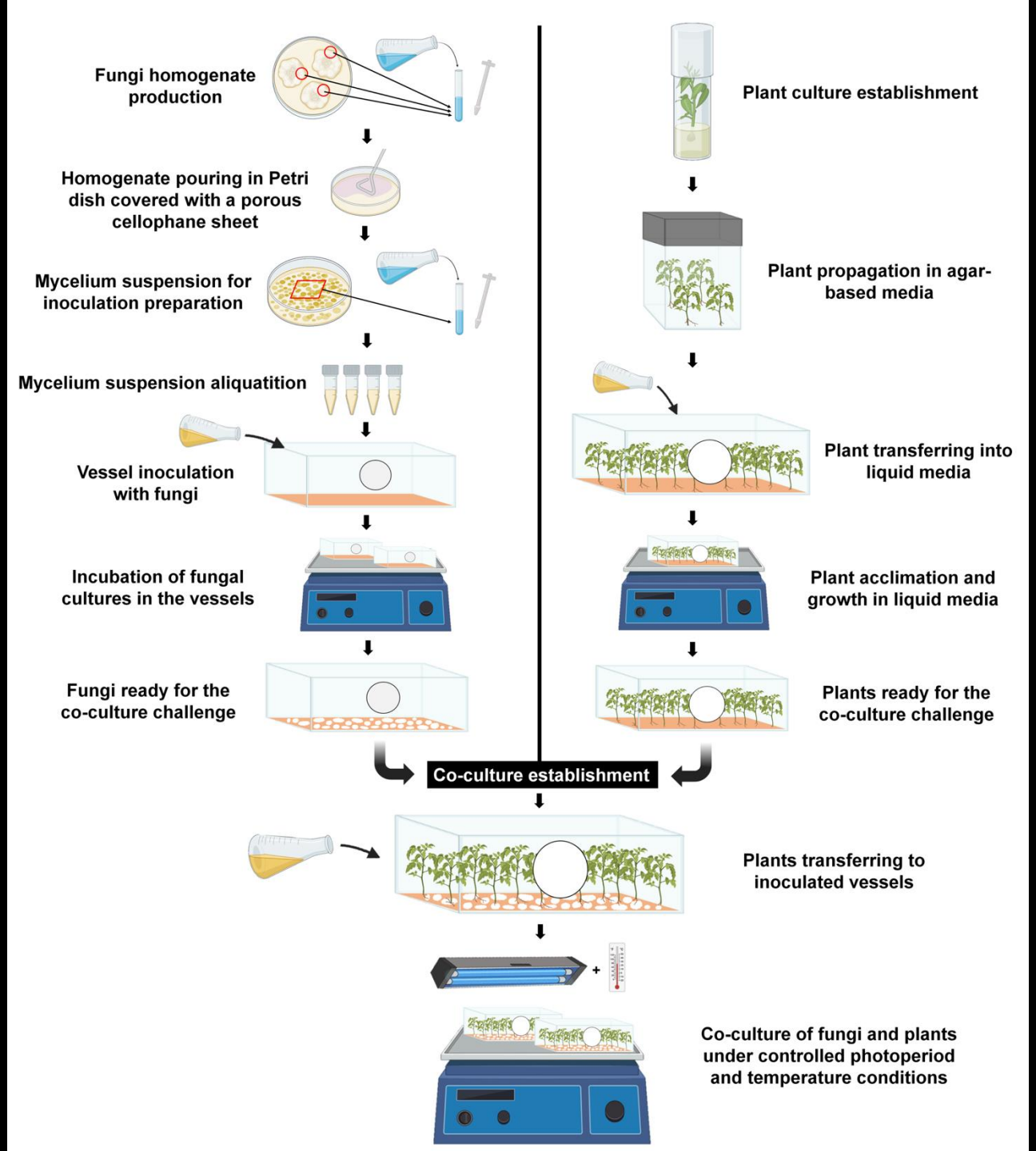
High Stain Examples cont.

DJ-8-65

Total vascular tissue area: 7328

Total stained area: 4823

Percent area stained: 65.82%


Fungi homogenate production
Homogenate pouring in Petri dish covered with a porous cellophane sheet
Mycelium suspension for inoculation preparation
Mycelium suspension aliquatition
Vessel inoculation with fungi
Incubation of fungal cultures in the vessels
Fungi ready for the co-culture challenge
Plant culture establishment
Plant propagation in agar-based media
Plant transferring into liquid media
Plant acclimation and growth in liquid media
Plants ready for the co-culture challenge
Co-culture establishment
Plants transferring to inoculated vessels
Co-culture of fungi and plants under controlled photoperiod and temperature conditions

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