

# Genetic and QTL mapping of Fusarium wilt (FOV) race 1 & race 4 resistance in cotton for breeding

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## • FOV Plant Symptoms:



- Wilting, yellowing, and necrosis of leaves.
- Internally, the xylem is dark brown [vascular root stain (VRS)].



# Plant Material & Tests

## Used two Mapping Populations:

- F<sub>2</sub> Pima-S7 x 'Acala NemX'
- RIL Upland TM-1 x Pima 3-79

## RIL Population evaluated in:

- Three greenhouse artificial inoculated tests ( 4 – 5 plants in a CRD)
- One known infested race 1 FOV field test ( 5 plants, two reps, RCBD)

# FOV race 1 assays

- **Used disease severity index (DSI) of leaves, and vascular stem and root staining [VRS (Scale 0 -5)].**
- **Disease was monitored on plants at different days after inoculation (dai) 9, 12, 14, 16, 19, 23, and 24.**
- **QTL used mean phenotypic scores of dai from the various experiments.**

# Greenhouse Test

Seeded RIL Population



race 1 FOV infection days after Inoculation (dai)

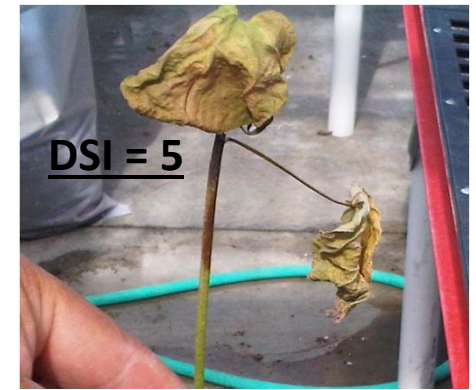
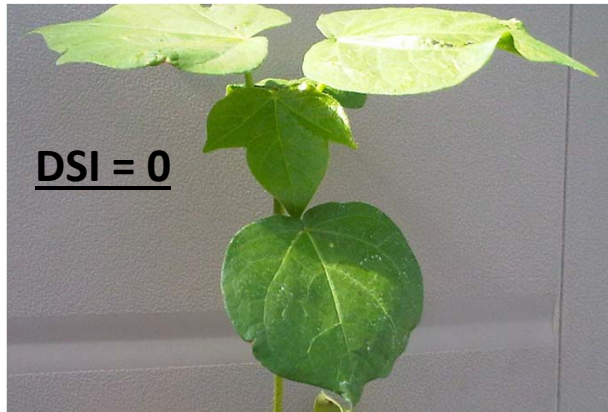


race 1 FOV Seedling  
Inoculation

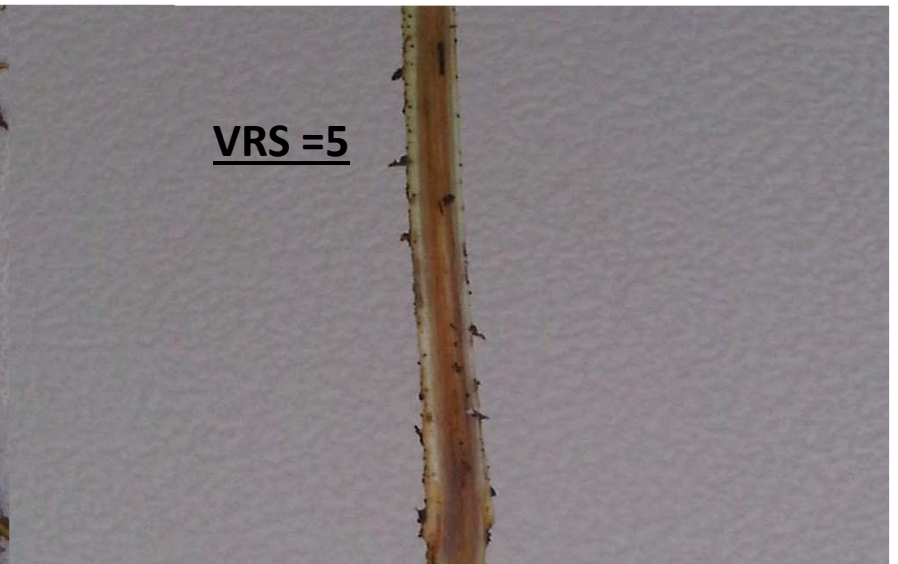
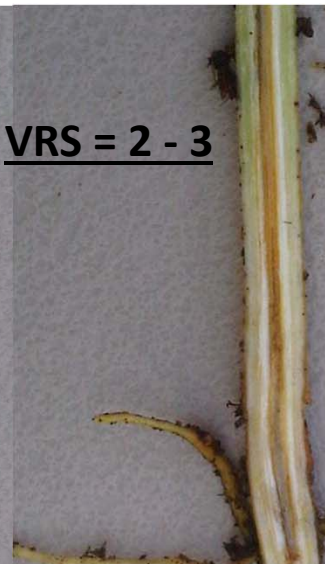


# Plant Infection

Disease severity index (DSI) of leaves, (scale 0 – 5)



Vascular stem and root staining (VRS)



# Field Assay



# Field Assay

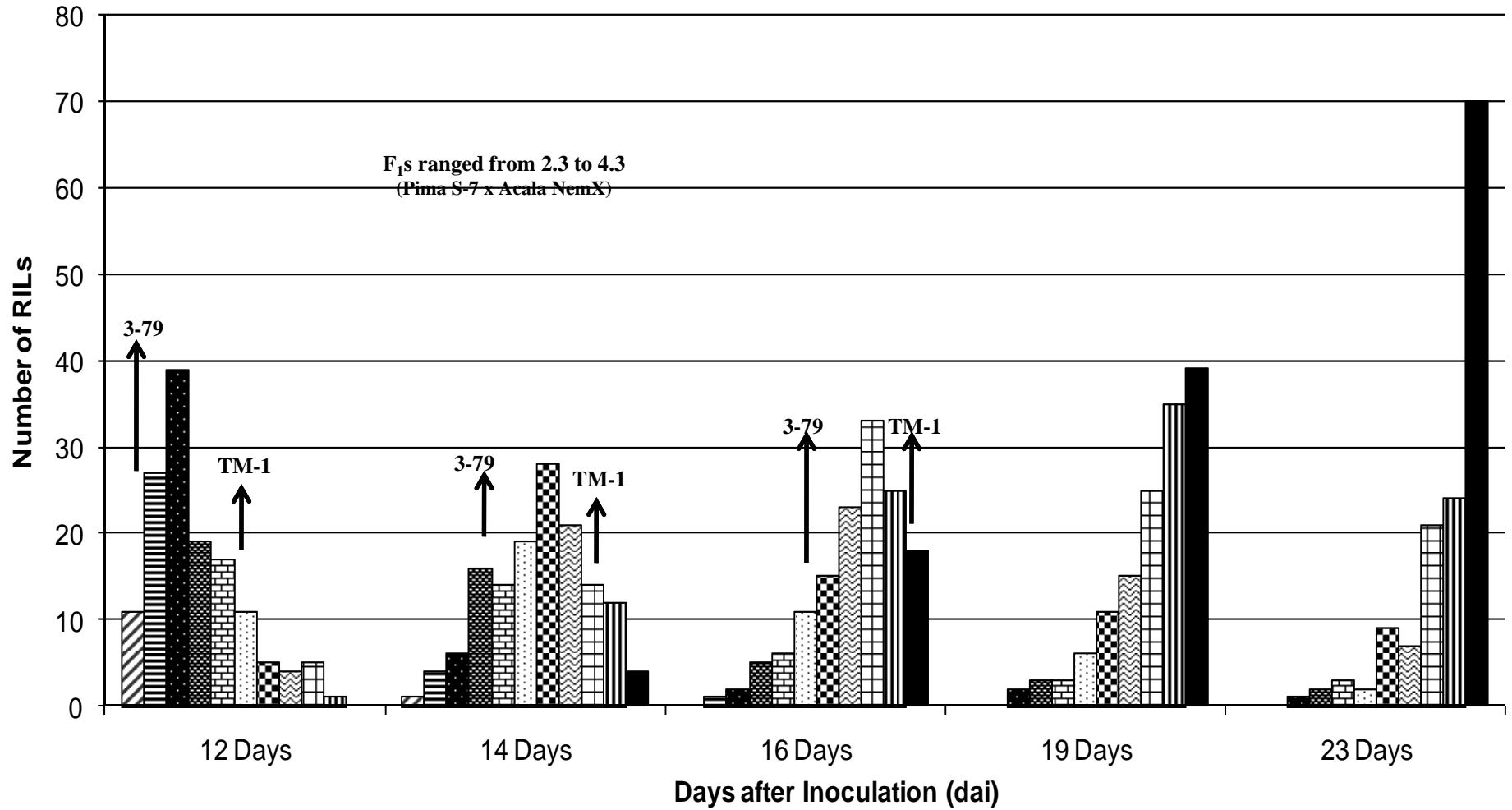




<b>Greenhouse Tests</b>		<b>RIL No</b>	<b>Mean</b>	<b>SD</b>
2006 Day 9	UC Riverside	139	2.84	1.37
2006 Day 14		139	3.68	1.23
2006 Day 23		139	4.56	0.88
2007 Day 12	UC Riverside	138	1.24	0.99
2007 Day 14		138	2.68	1.14
2007 Day 16		138	3.48	1.00
2007 Day 19		138	3.89	0.95
2007 Day 23		138	4.24	0.90
2008 Day 24	UC Kearney	137	2.52	1.08
<b>Field Test Shafter CA</b>		<b>RIL No</b>	<b>Mean</b>	<b>SD</b>
2007 Day 67 after planting		113	1.61	0.71
2007 Day 67 % Survival		113	85.74	13.37
2007 Day 146 % Survival		113	27.39	19.36

### Disease Severity Index (DSI)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0



# Race 1 FOV Inheritance in Pima by Acala and Upland by Pima Cottons

Genotype/ Population	Expected Ratios R:H:S & R:S	Observed ratio R:S	$\chi^2$	P-value
<b><u>Parents</u></b>				
Pima S7	All R	38:5		
Acala NemX	All S	5:39		
$\underline{F}_1$	All S	6:60		
$\underline{F}_2$	33:100 (1:3)	31:102	0.203	NS
<b><u>Parents</u></b>				
Upland TM1	All S	0:5		
Pima 3-79	All R	4:1		
RIL (2007) Day 14	41:97 (1:3)	35:103	1.379	NS
	(1:1)	35:103	18.03	0.001
Upland TM1	All S	0:4		
Pima 3-79	All R	1:3		
RIL (2008) Day 24	52:78 (1:3)	34:103	17.83	0.001
	52:78 (1:1)	68:69	4.94	0.05

# SSR Markers and QTLs for FOV race 1

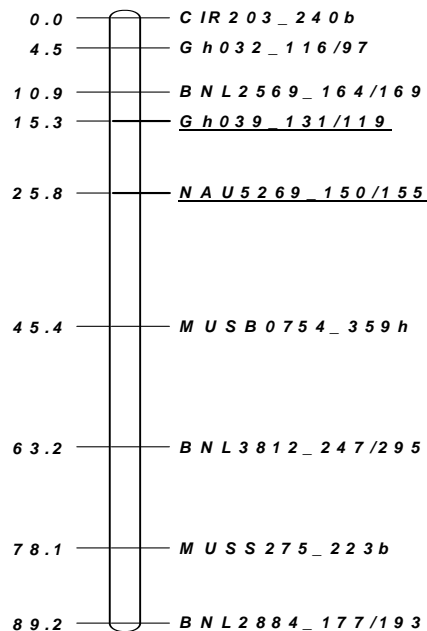
## Resistance based on Vascular Stem and Root Staining

Locus	TM1 allele Mean	3-79 allele Mean	K*†	<i>P value</i>
<b>24 SSRs Identified</b>	<b>2.88 - 3.10</b>	<b>1.70 – 2.35</b>	<b>8.2 – 22.5</b>	<b>0.01</b>

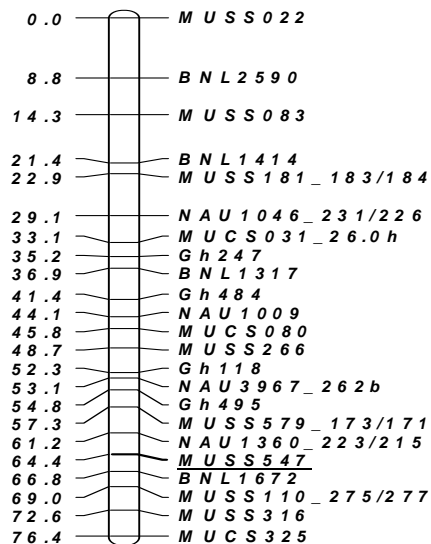
**Nine Chromosomes: 01, 06, 08, 09, 11, 12, 15, 16, and 19**

†Kruskal-Wallis analysis (K\*) test - nonparametric 1-way ANOVA

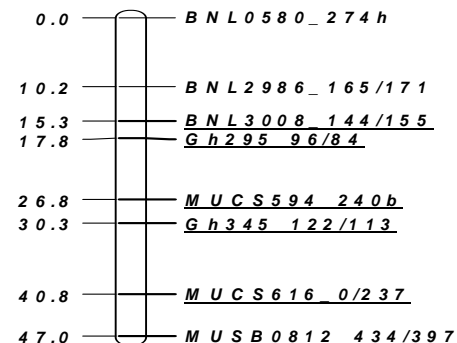
### Chromosome 6



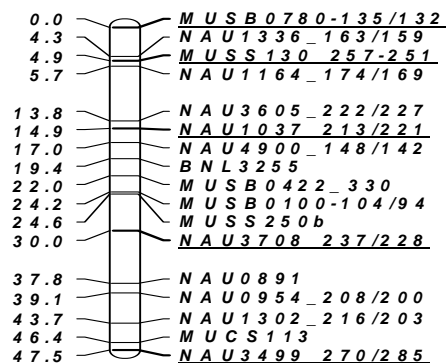
### Chromosome 9



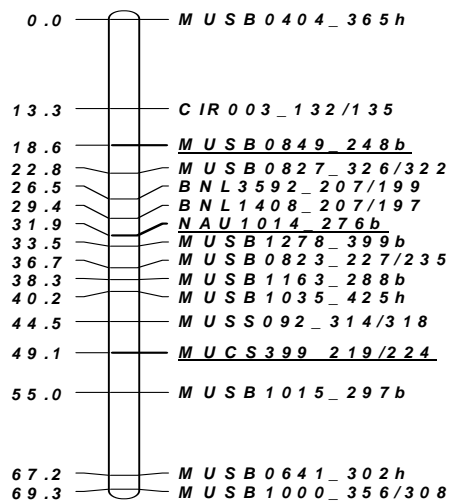
### Chromosome 16



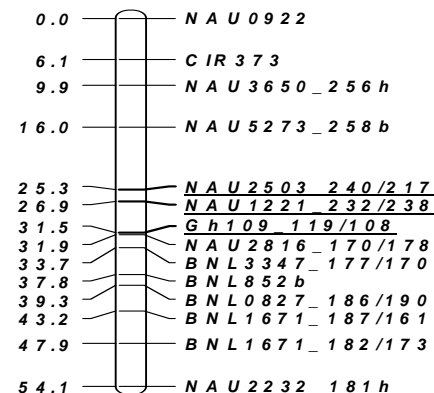
### Chromosome 8



### Chromosome 11

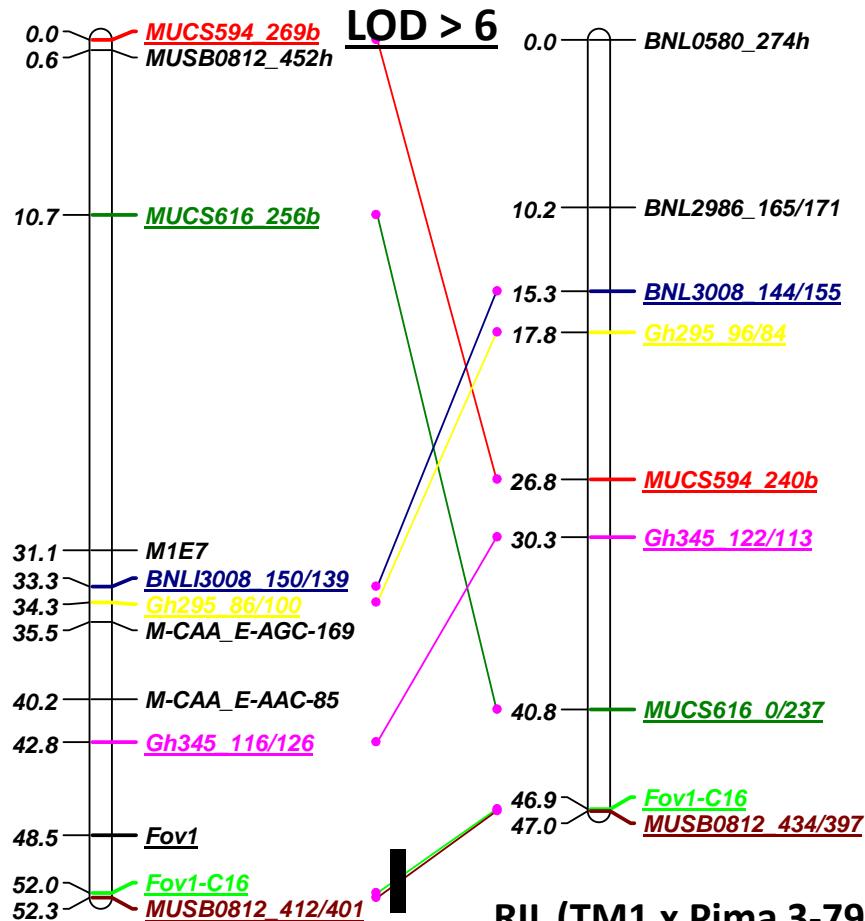


### Chromosome 19



QTL =

## Chromosome 16



QTL explained 27% of DSI variation with an additive effect of 0.77.

QTL explained 31% of VRS variation with an additive effect of 0.72.

## Major QTLs were detected :

- ❖ **QTL on chromosome 6** explained from 8.0 to 22.3% of variation in DSI with additive effect ranging from 0.33 to 0.51.
- ❖ **QTL on chromosome 8** explained from 8.8 to 10.6% of DSI variation with additive effect ranging from 0.31 to 0.33.
- ❖ **QTLs on chromosome 11** (two) explained from 9.0 to 14.2% of DSI variation with an additive effect averaging 0.39 – 0.43.
- ❖ **QTL on Chromosome 19** explained 13% of DSI variation with an additive effect of 0.46.

# Mapping Fusarium wilt (FOV) race 1 resistance summary

- A set of 24 SSR markers indicated gene interactions and inheritance from nine cotton chromosomes, with major QTLs detected on five. Breeding targets.
- The single recessive locus (*Fov1*), previously identified in F<sub>2</sub> (Pima-S7x NemX) mapped to chromosome 16 and co-located with the *Fov1* and QTL in the TM1 x 3-79 RIL. Breeding target.

Breeding for Race 4  
Fusarium wilt (FOV)  
Disease Resistance



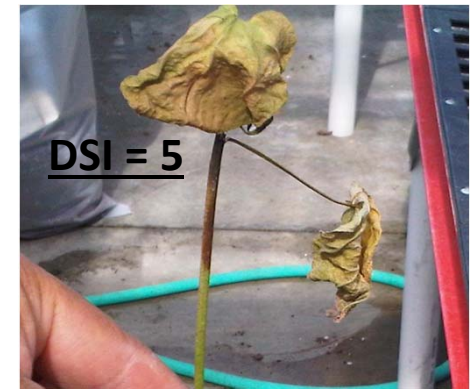
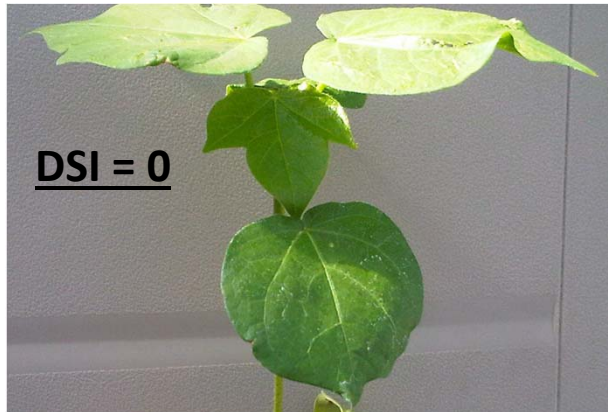
# Fusarium wilt (FOV) Symptoms



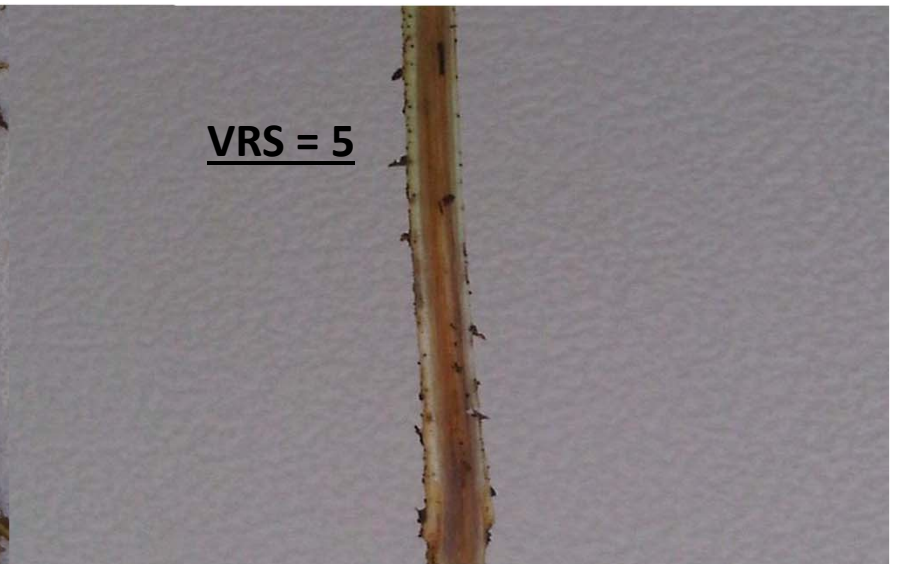
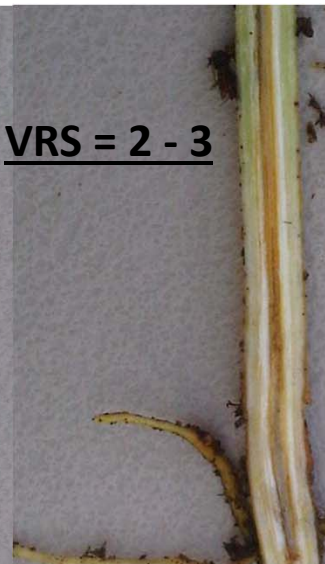
# Plant Infection

Resistant < 2.0 & Susceptible > 2.0

Disease severity index (DSI) of leaves, (scale 0 – 5)



Vascular stem and root staining [VRS (scale 0 - 5)]



# Race 4 FOV Disease Resistance

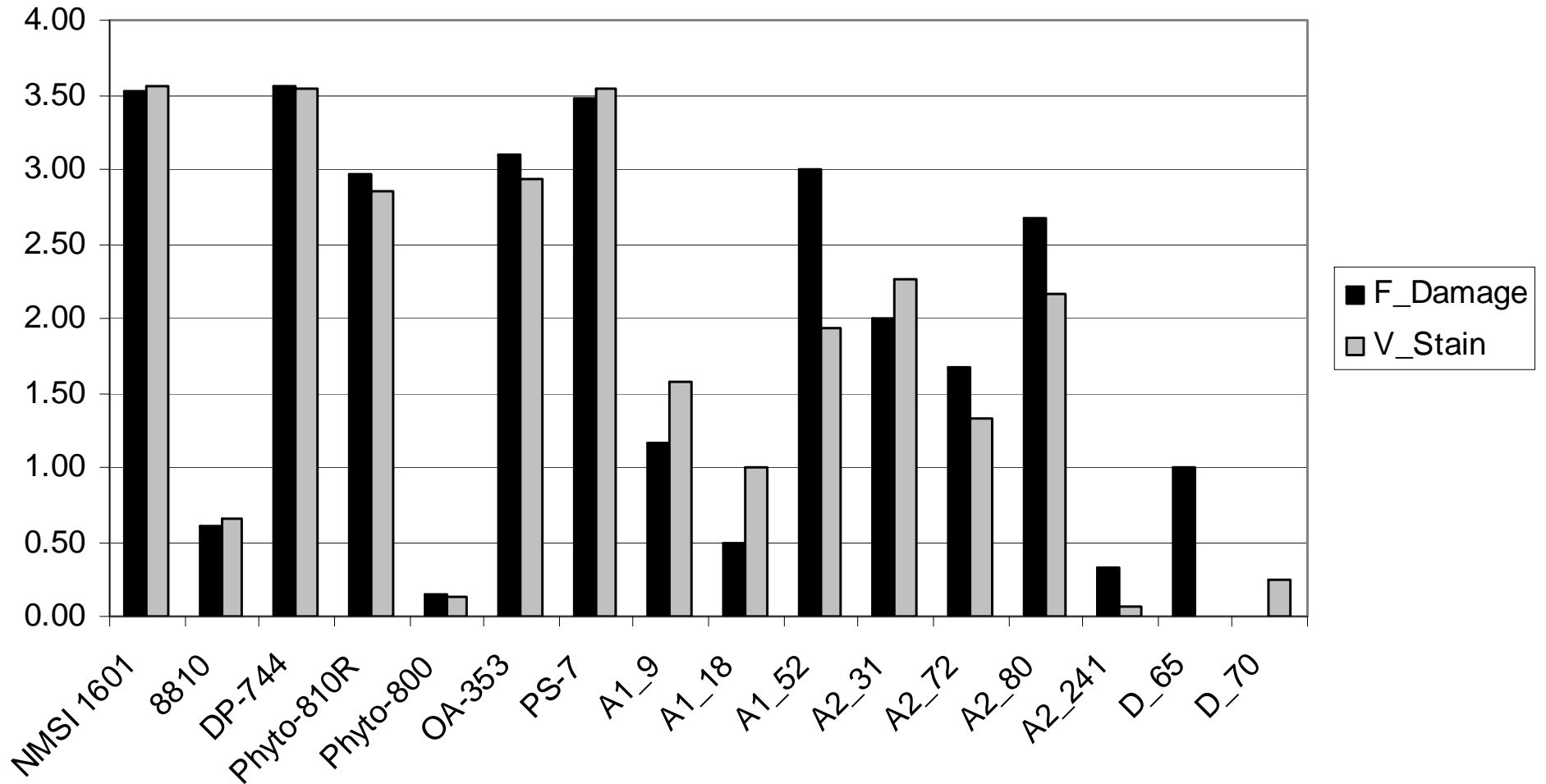
Resistant & Susceptible



Resistant & F<sub>1</sub> Progeny

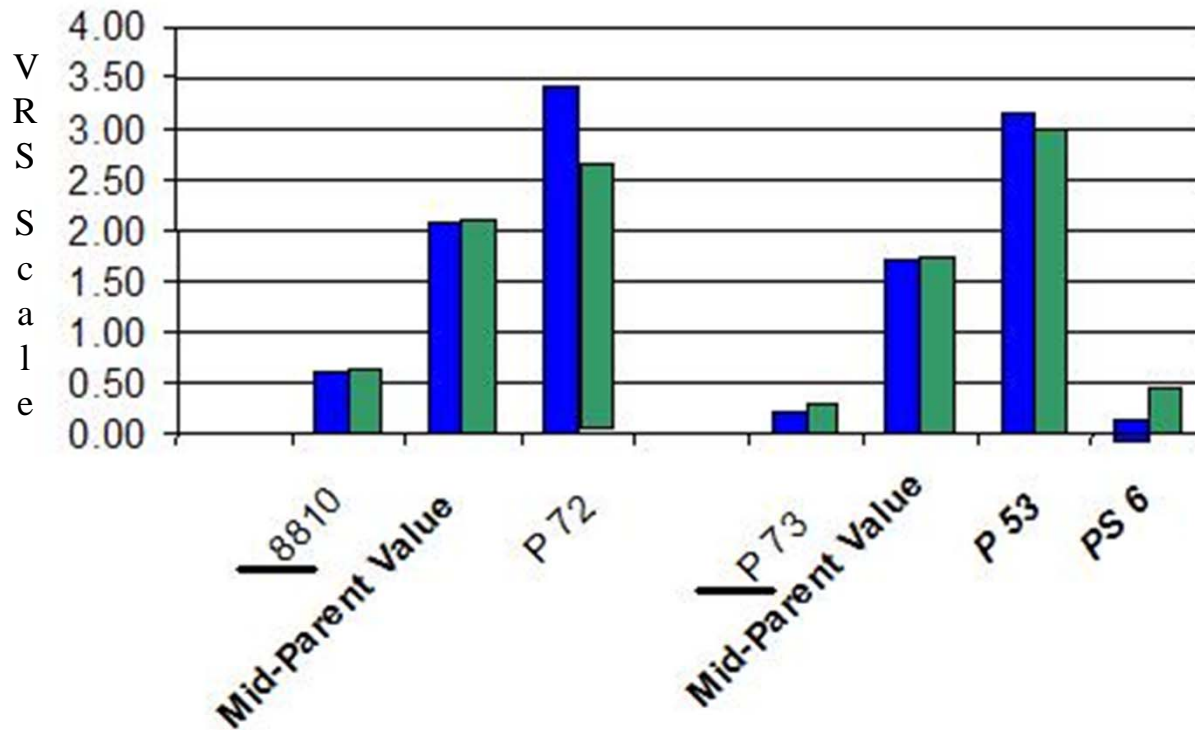


# Identification of Resistant Sources from Early Evaluations Pima Cottons and Accessions



# Race 4 FOV Inheritance Ulloa et al (2006)

Pima by Pima Crosses



PS:  $R^2 = 0.56$  &  
 $h^2 = 0.61$

F\_Damage:  $R^2 = 0.58$  &  
 $h^2 = 0.64$

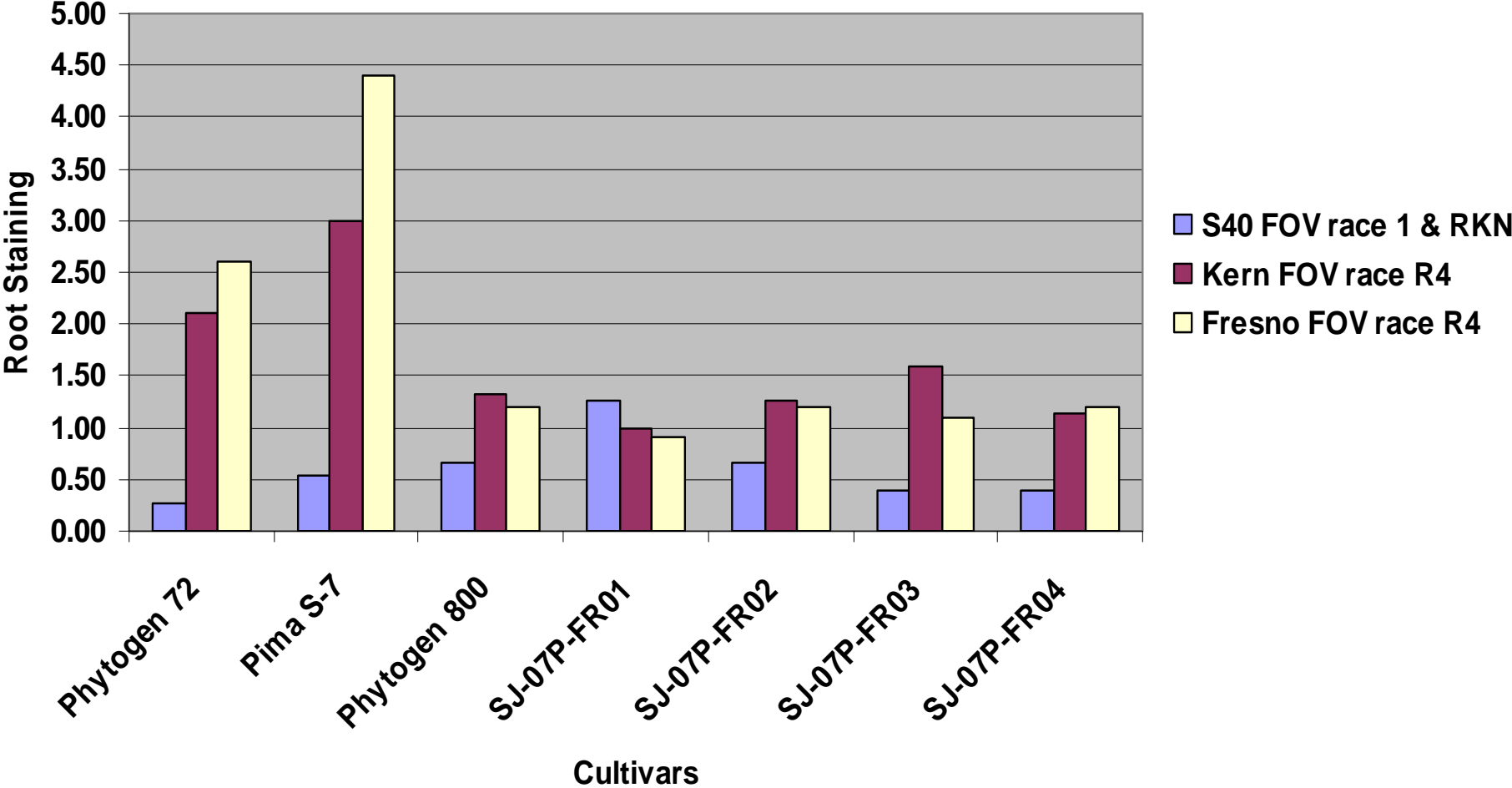
VRS:  $R^2 = 0.63$  &  
 $h^2 = 0.70$

8810 Developed from P 73 x P 72

P 73 Developed from P 53 x PS 6

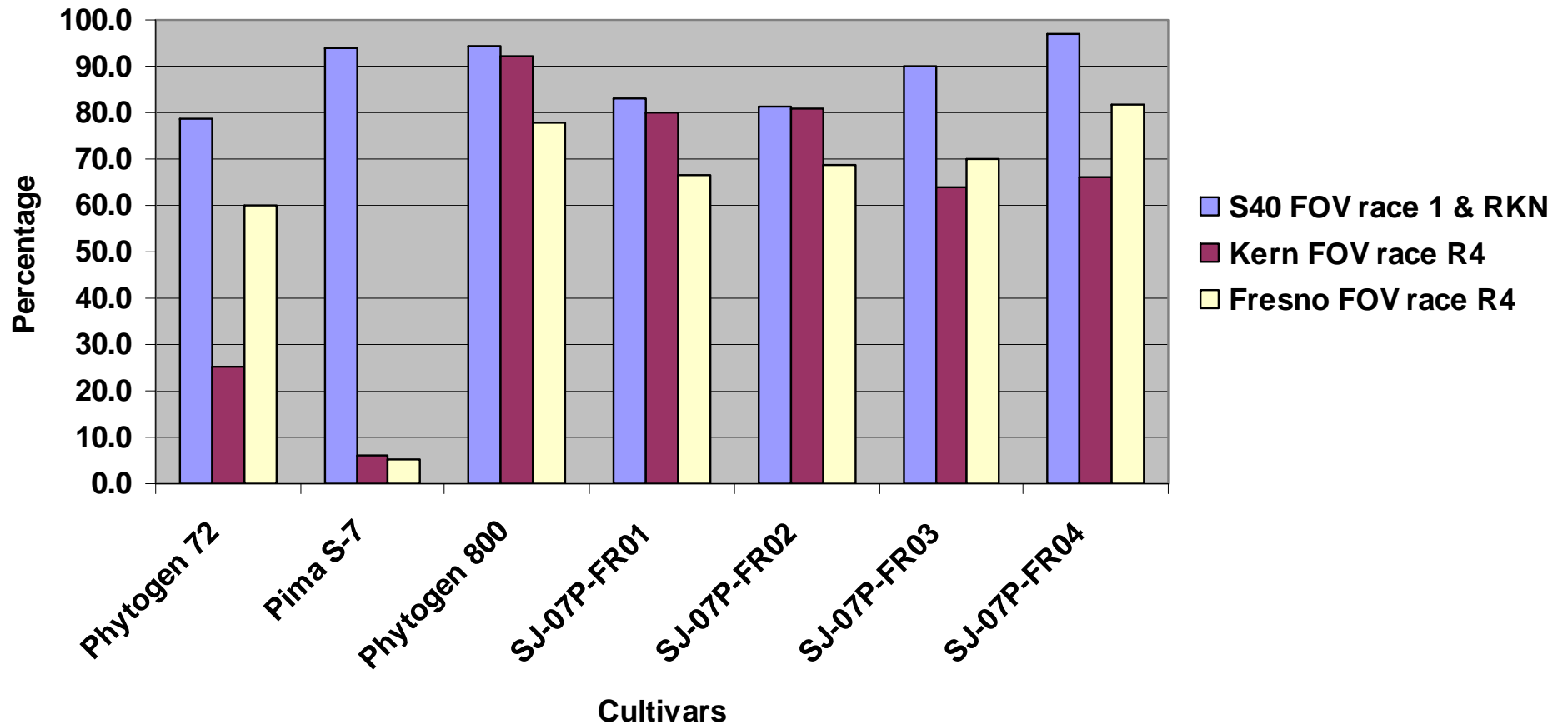
# Root top Vascular Stain Evaluation

Vascular Root Staining Fusarium wilt (FOV)



# Plant Percentage Survival

Percentage Survival Rate Fusarium wilt (FOV)



## Four Pima Germplasm Lines Jointly Released by USDA-ARS, Univ. of California, & NMSU

➤ **SJ-07P-FR01**

➤ **SJ-07P-FR02**

➤ **SJ-07P-FR03**

➤ **SJ-07P-FR04**

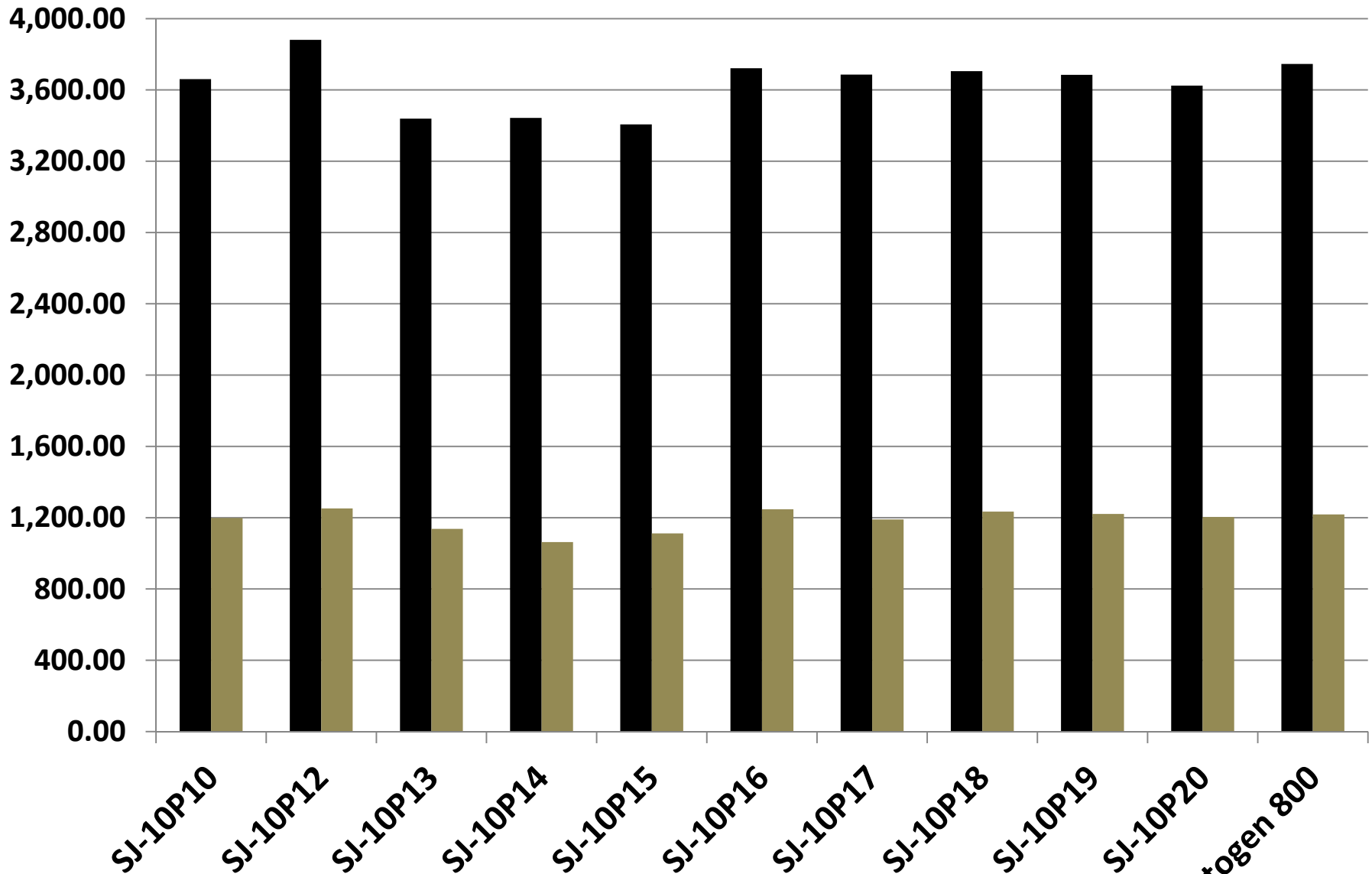
➤ Continuing efforts for releasing additional germplasm with improved Yield, Fiber, and Pest Resistance



LB per Acre

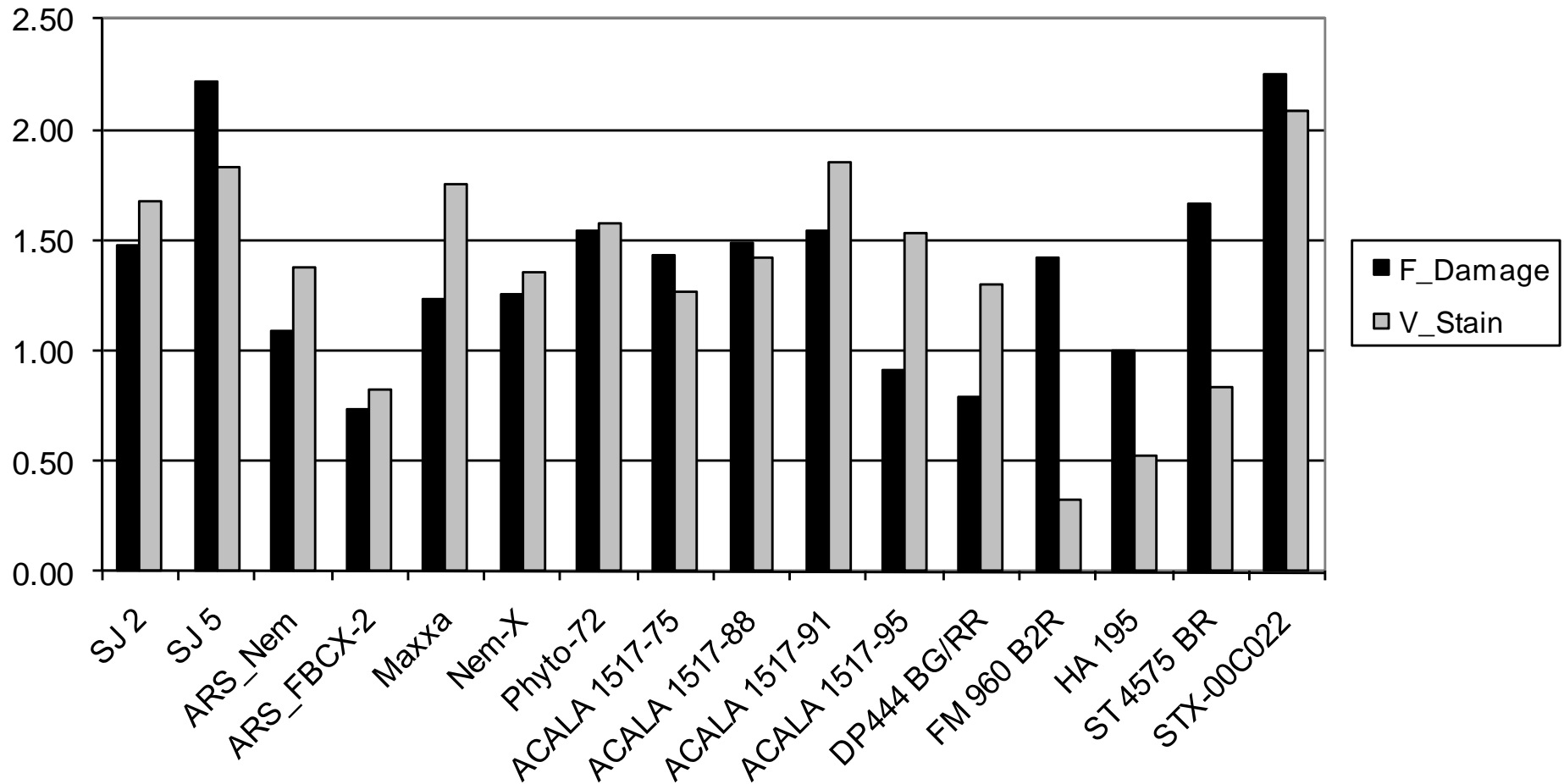
■ Seed Cotton

■ Lint Yield



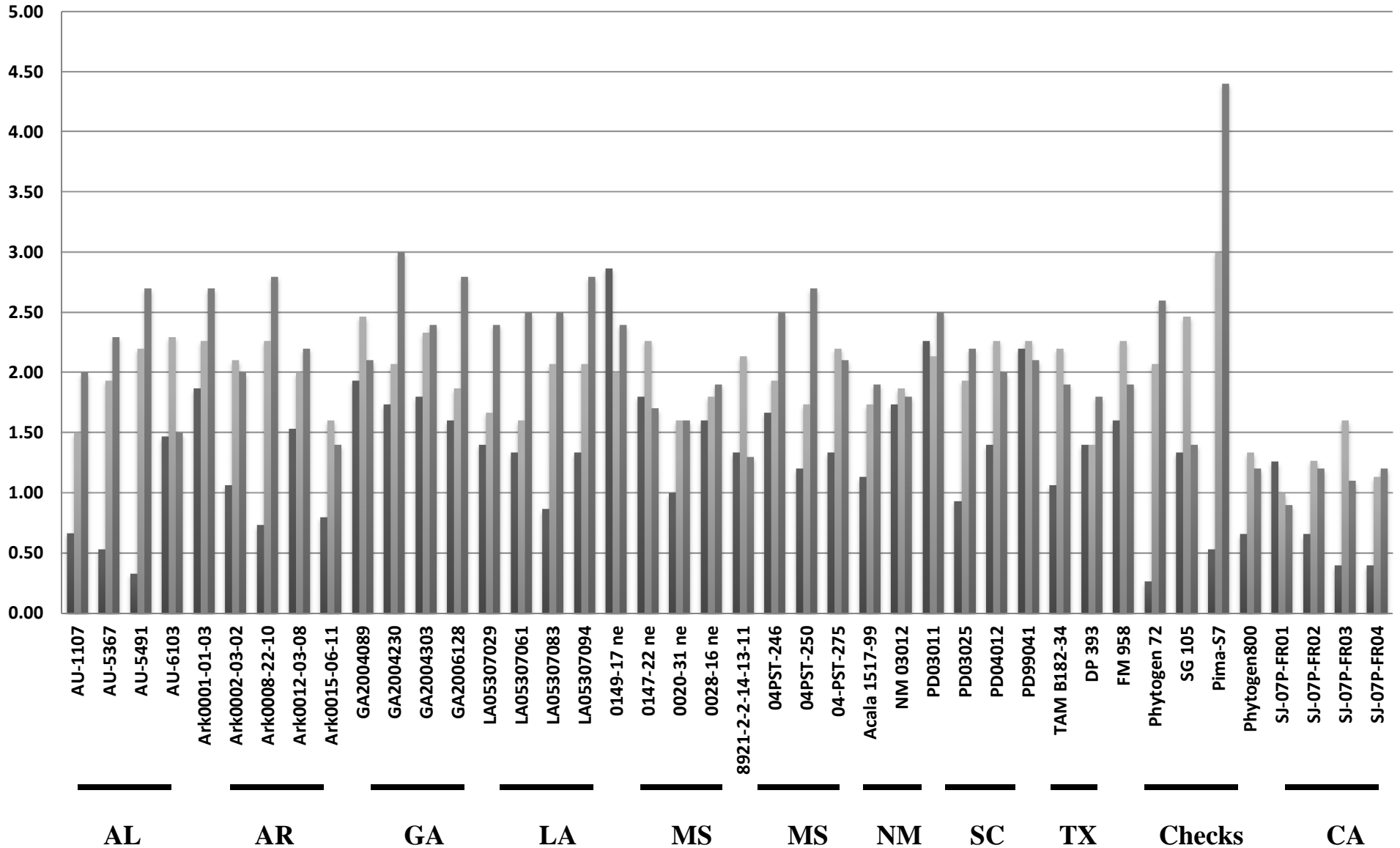
Pima New Releases USDA-ARS and Univ. of CA. (R. Percy, R. Hutmacher, S. Wright, and M. Ulloa)

# Identification of Resistant Sources from Early Evaluations Acala and Upland Cottons



# Vascular Root Staining [scale 0 = no staining to 5 = plant death]

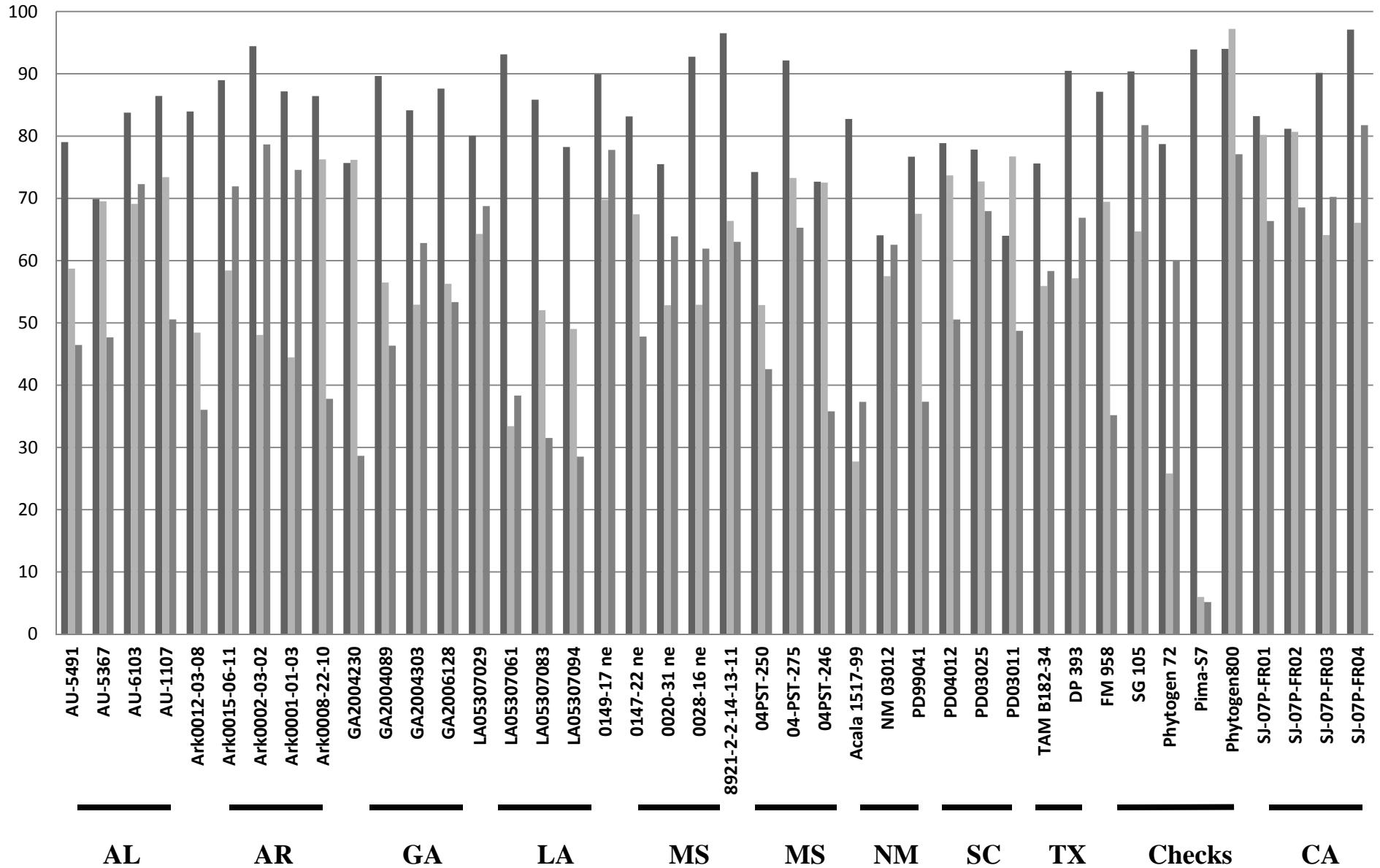
■ Shafter FOV race 1 & RKN   ■ Kern FOV race 4   ■ Fresno FOV race 4



RBTN Entries in 2008

# Percent of Plant Survival [scale 0 to 100 %]

■ Shafter FOV race 1 & RKN    ■ Kern FOV race 4    ■ Fresno FOV race 4



RBTN Entries in 2008

# QTL mapping & breeding resistance to FOV race 4

- 1200 genome-wide markers analyzed
- Populations from Upland-by-Upland, Upland-by-Pima, and Pima-by-Pima cottons.

# Parents, Crosses, & Progeny for FOV & Root-knot nematode Research

	<b>FBCX-2</b>	<b>Shorty</b>	<b>PS 6</b>	<b>3-79</b>	<b>DP 744</b>	<b>Uzbekistan</b>	<b>A<sub>1</sub></b>
<b>Nem_ARS</b>	X		X	X	X	X	
<b>FBCX-2</b>		X	X		X		
<b>Shorty</b>	X		X		X	X	
<b>PS 6</b>	X	X			X	X	
<b>PS 7</b>	X	X	X	X	X	X	
<b>DP 744</b>	X	X	X	X			
<b>TM 1</b>			X	X		X	
<b>A<sub>2</sub></b>							X

# FOV 4 Screens for VSR in F2 and RILs

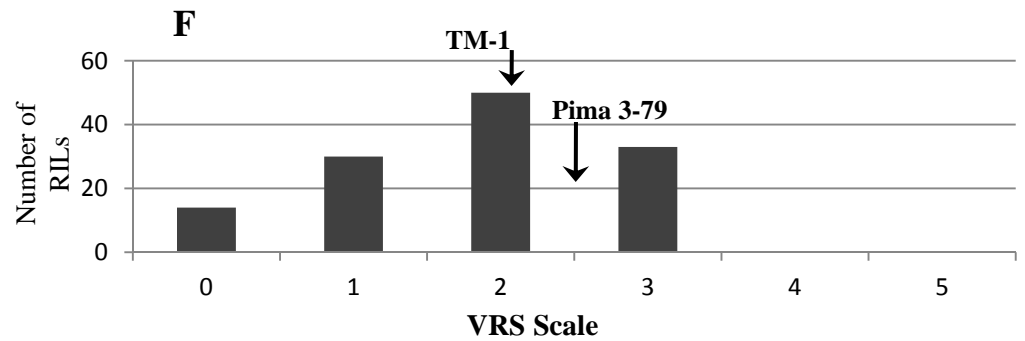
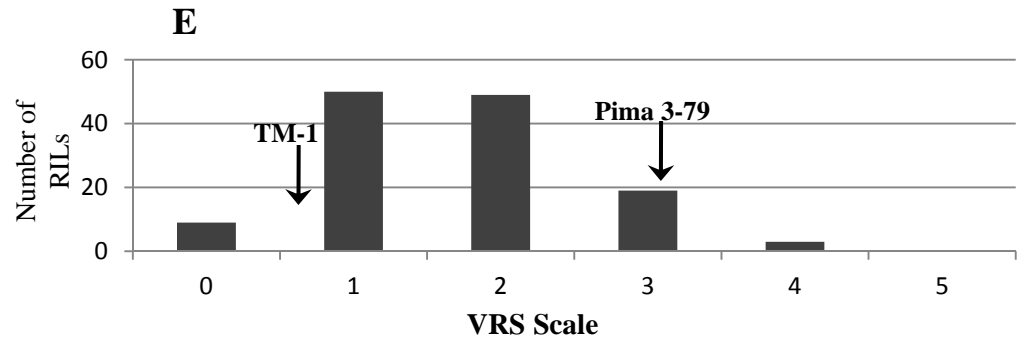
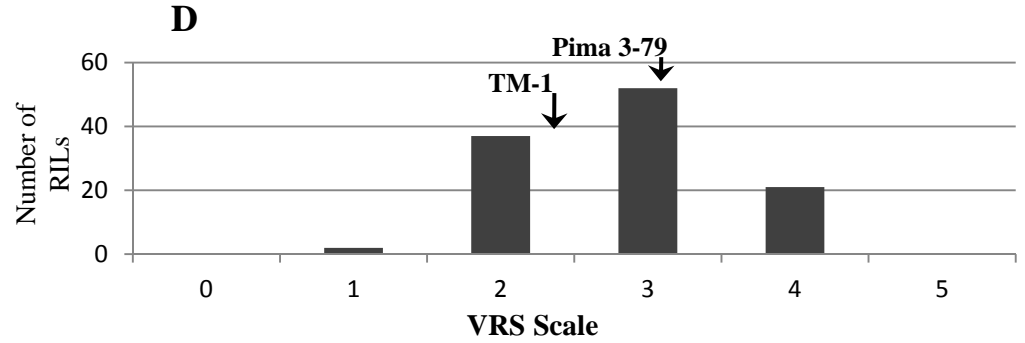
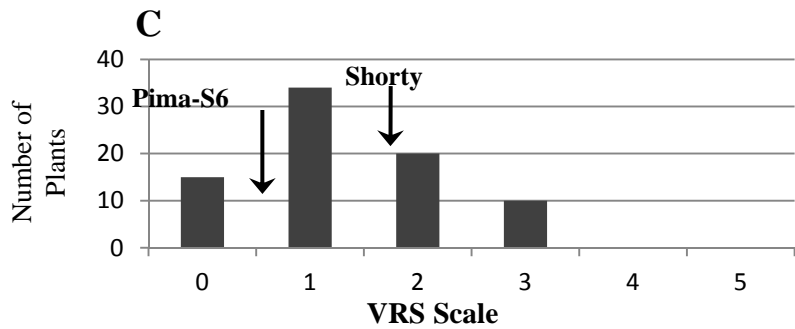
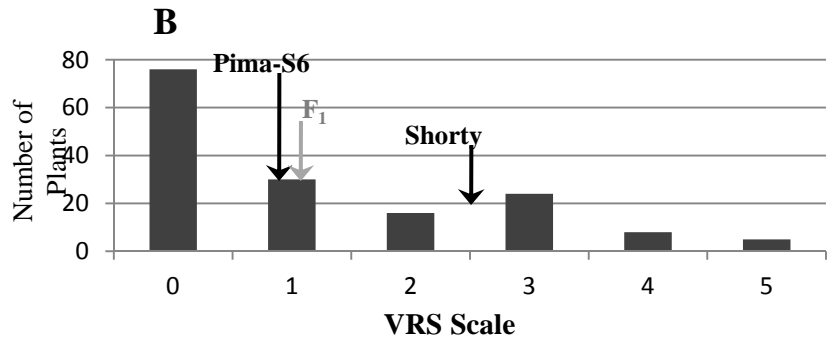
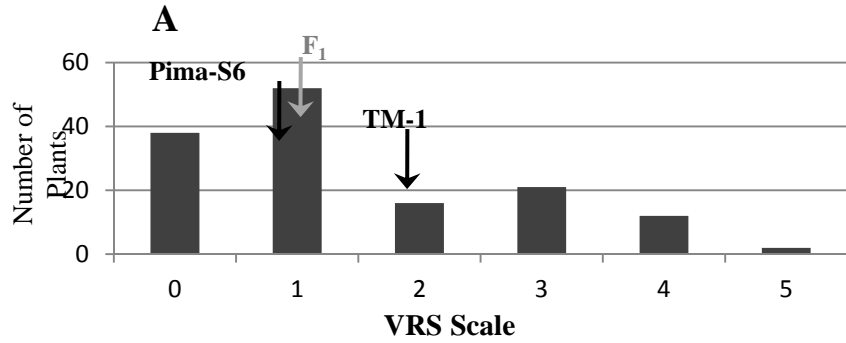
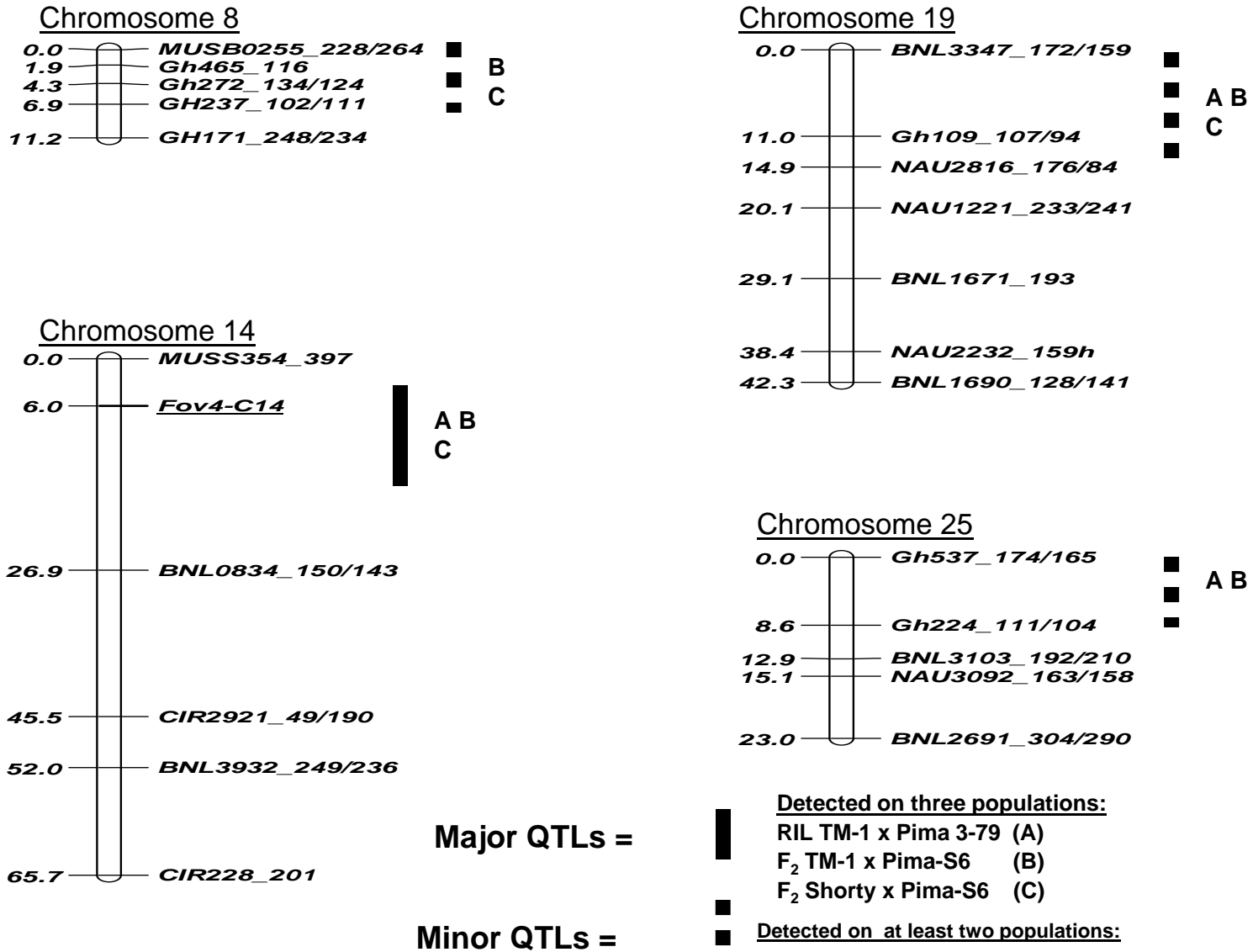


Fig 2





# Different QTL for Resistance to Race 1 and Race 4

## Major QTL for FOV4

❖ QTL on chromosome 8 explained from 5 to 19% of variation in DSI & VRS.

❖ QTL on chromosome 14 explained from 18 to 80% of variation in DSI with additive effect ranging from 0.36 to 1.58; and 76% of variation for DSI and 1.10 additive effect.

❖ QTL on chromosome 19 explained 72% of VRS variation with dominant effect ranging of 1.80.

❖ QTLs on chromosome 25 explained from 15% of VRS variation and 1.80 additive effect .

## Major QTL for FOV1

❖ QTL on chromosome 16 explained from 27 to 31% of variation in DSI & VRS with additive effect ranging from 0.72 to 0.77.

❖ QTL on chromosome 6 explained from 8.0 to 22.3% of variation in DSI with additive effect ranging from 0.33 to 0.51.

❖ QTL on chromosome 8 explained from 8.8 to 10.6% of DSI variation with additive effect ranging from 0.31 to 0.33.

❖ QTLs on chromosome 11 (two) explained from 9.0 to 14.2% of DSI variation with an additive effect averaging 0.39 – 0.43.

❖ QTL on Chromosome 19 explained 13% of DSI variation with an additive effect of 0.46.