

UNDERSTANDING DROUGHT TOLERANCE AND BREEDING SOYBEANS FOR SUSTAINABLE YIELD UNDER DROUGHT STRESS

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Drought inevitably presents a challenge to production agriculture. This challenge is increasing as a result of continuing global climate change and scarcity of water resources. The 'Team Drought', consisting of scientists from the U.S. Department of Agriculture and several universities in the U.S., has tackled the drought problem in soybean production using multidisciplinary approaches for almost two decades. Team Drought's research, funded by farmers through the United Soybean Board, addresses the complexities of drought tolerance by dissecting the physiology and genetics of yield potential under drought stress. A critical component of success was the survey of the USDA Soybean Germplasm Collection to identify plant introductions carrying favorable traits associated with drought tolerance and subsequent utilization in breeding for sustainable yields under drought stress. Two rare slow-wilting genotypes, PI 416937 and PI 471938, were discovered as having unusual physiology and genetics for drought tolerance. Slow wilting in PI 416937 appeared to be associated with an early-season soil water-conserving mechanism coupled with a restricted transpiration by partial stomata closure. PI 416937 carries five quantitative trait loci (QTL) for slow wilting, five QTL for prolific rooting, and a major QTL for limited leaf hydraulic conductance, all of which are associated with drought tolerance. PI 471938 has three QTL for slow wilting, although the mechanism is not yet clear, with each contributing to a 136 kg/ha yield increase under drought stress. Two breeding lines derived from these slow wilting sources have shown excellent yield potential, equal to or greater than that of the check cultivars in the USDA Uniform Tests across multiple environments. Prolonged nitrogen (N₂) fixation under drought stress was discovered in Jackson, PI 227557, and PI 471938. N₂ fixation activity in these genotypes was less sensitive to drying soil, thus, provides a yield advantage under water deficit conditions. Six breeding lines were developed with sustained N₂ fixation in a drying soil that have yields competitive with checks in irrigated conditions and have a 10-15% yield advantage over checks in drought stress conditions. Diverse soybean germplasm was also screened for fast and/or deep rooting characteristics.

Soybean Drought Tolerance Research in the U.S.

Pengyin Chen

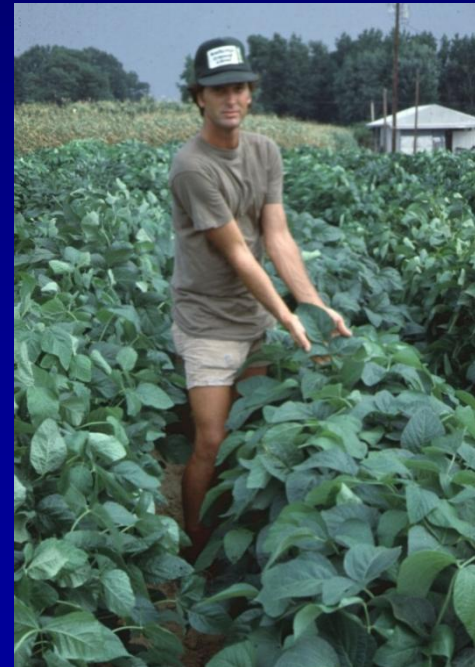
University of Arkansas

Drought ?!!



Research Goal

- Understanding drought tolerance
- Breeding for sustainable yield under drought stress

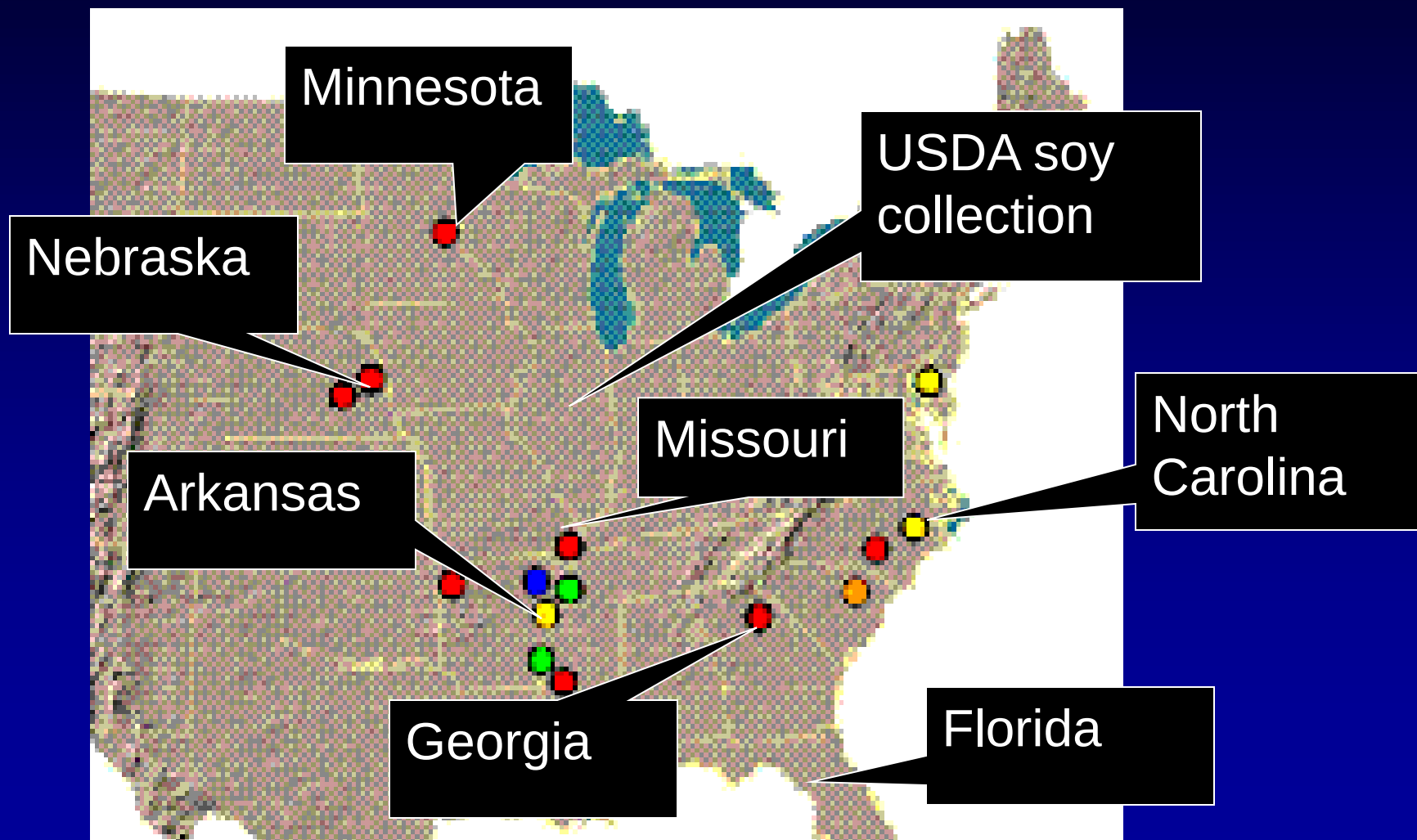


Research Contributions

- [**USDA-ARS**
- [**North Caroline State Univ.**
- [**Univ. of Arkansas**
- [**Univ. of Georgia**
- [**Univ. of Missouri**
- [**Univ. of Minnesota**
- [**Univ. of Nebraska**
- [**Univ. of Florida**



Geographic Coverage

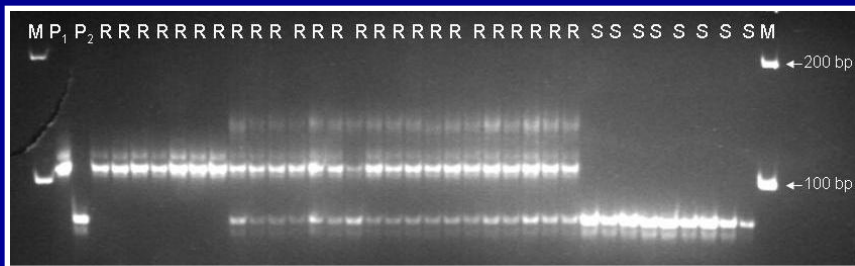


Team Drought



Research Approach

- Physiologic
- Genetic
- Molecular
- Agronomic
- Breeding



Traits of Interest

- Slow-wilting
- N₂-fixation
- Rooting
- Yield



Other Related Traits

- ☐ Water use efficiency
- ☐ Radiation use efficiency
- ☐ Leaf stomatal response to dry air
- ☐ Aquaporin protein vs. water transport
- ☐ Response to vapor pressure deficit & temperature
- ☐ Volumetric soil water content
- ☐ Shoot ureide concentration
- ☐ Acetylene reduction activity

**Early 1990's
North Carolina**

**Tommy
Carter**

Essex

PI 416937

Discovery of the Slow-Wilting Trait



Minnesota

Slow
wilting
type





Fast
Wilting

Slow
Wilting

Nebraska

Slow Wilting in Arkansas



Slow Wilting

Fast Wilting

Drought in Georgia



Slow Wilting Discovery

Carolina

5 Asian types

Minnesota

6 (more) Asian types

Nebraska

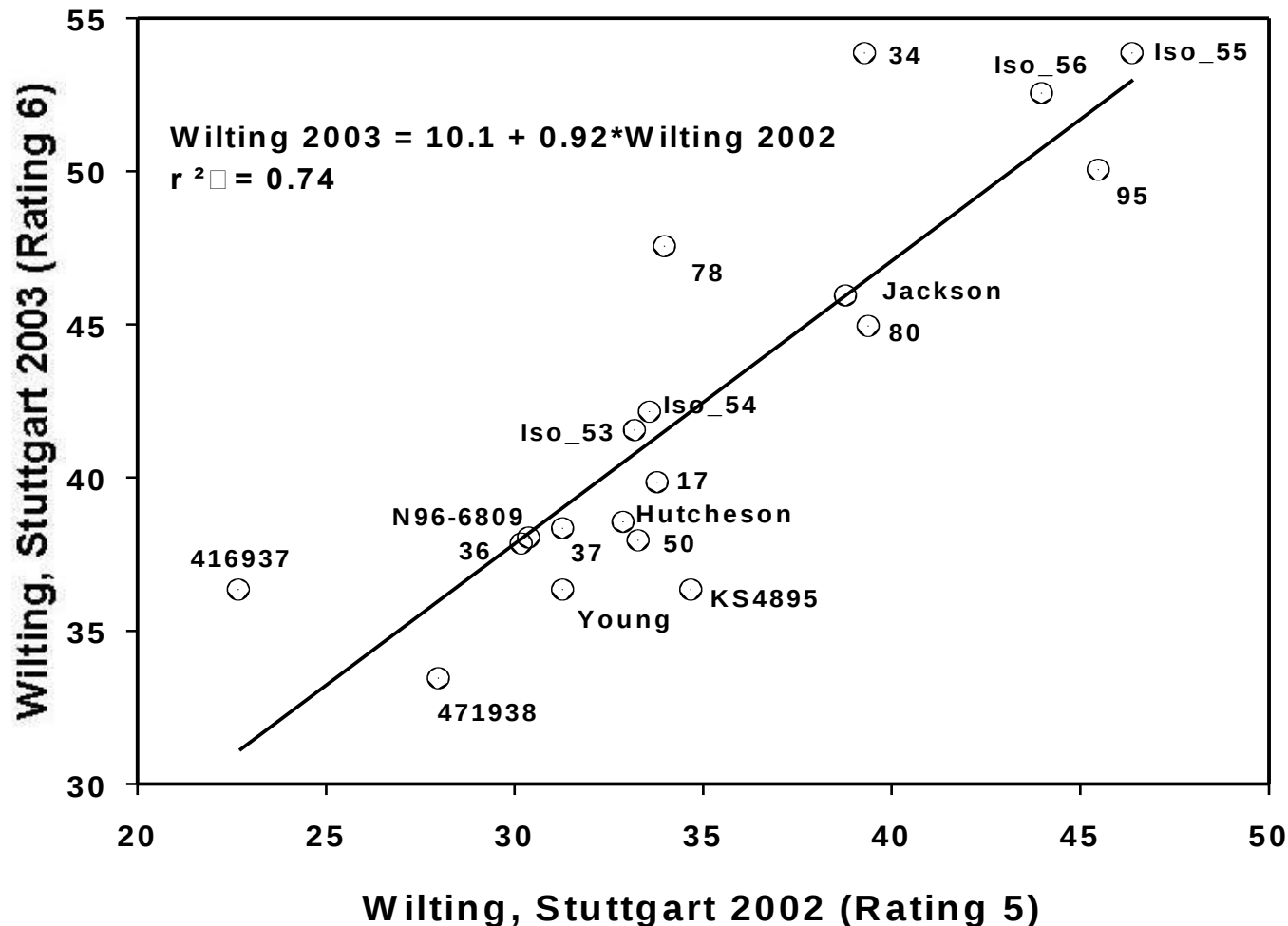
10 (more) Asian types

Arkansas

2 U.S. types

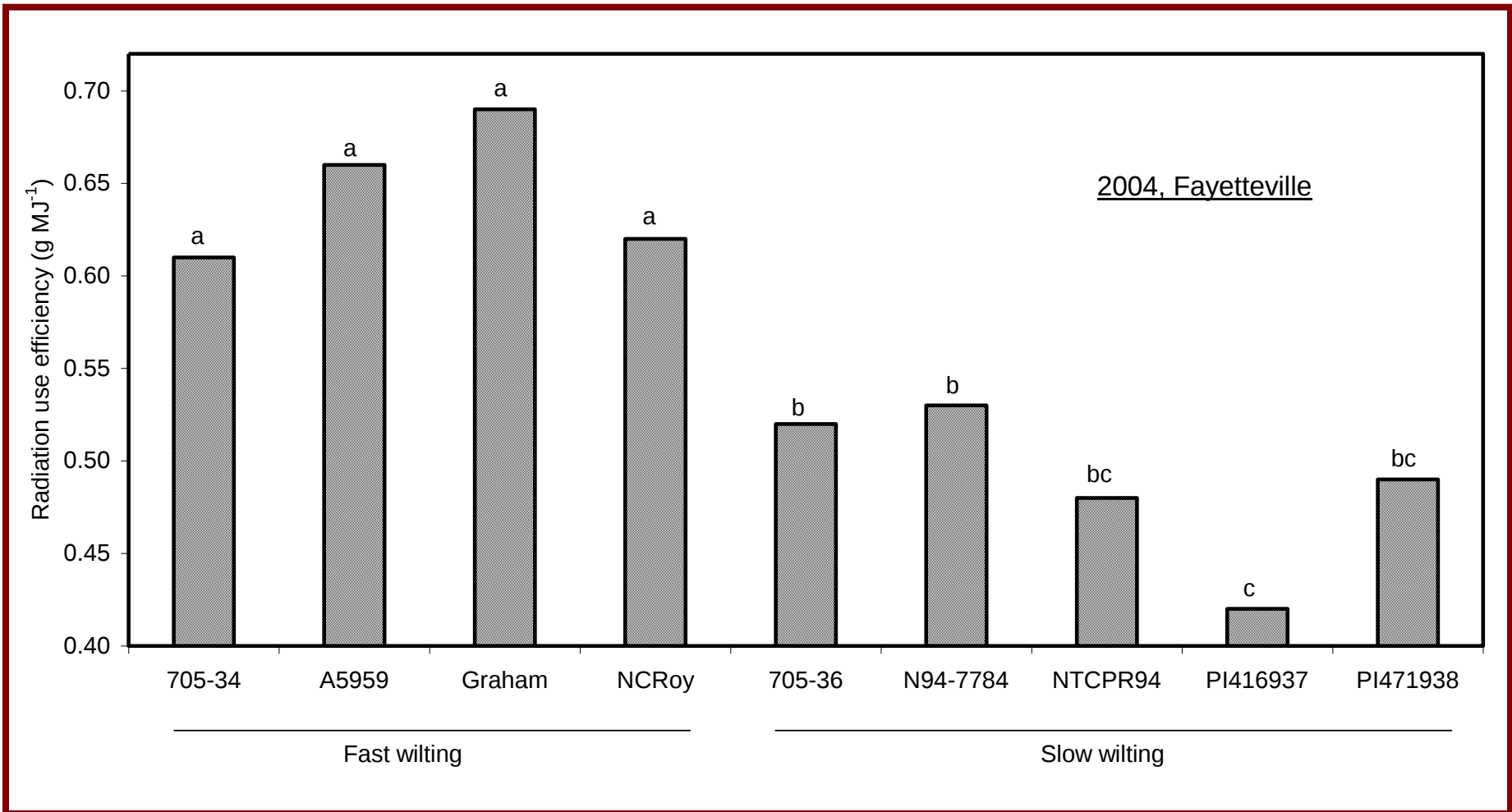


Slow Wilting → A Stable Trait



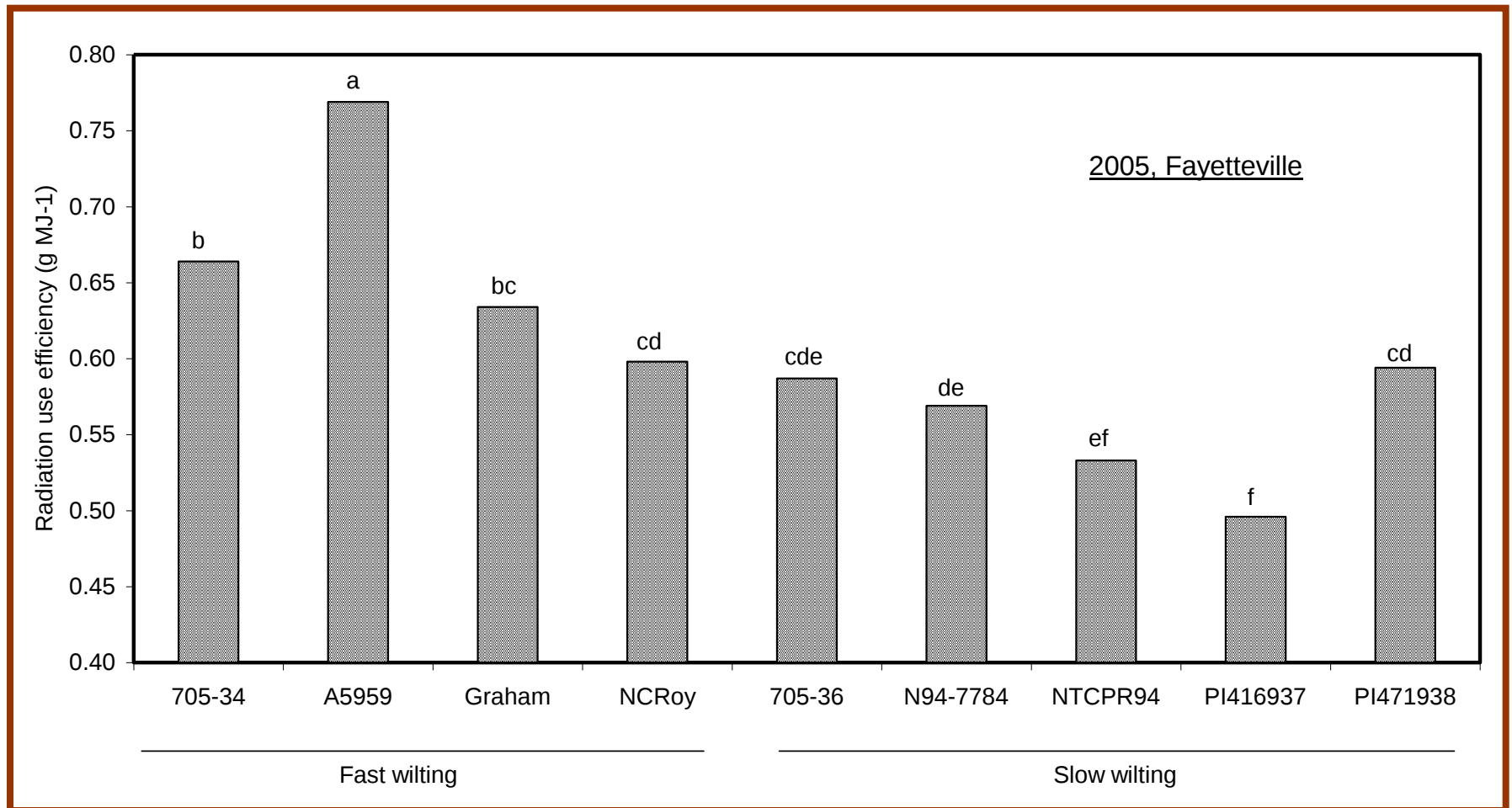
King, Purcell, and Brye. 2009. Crop Sci. 49: 290-298.

Wilting vs. Radiation Use Efficiency (RUE)



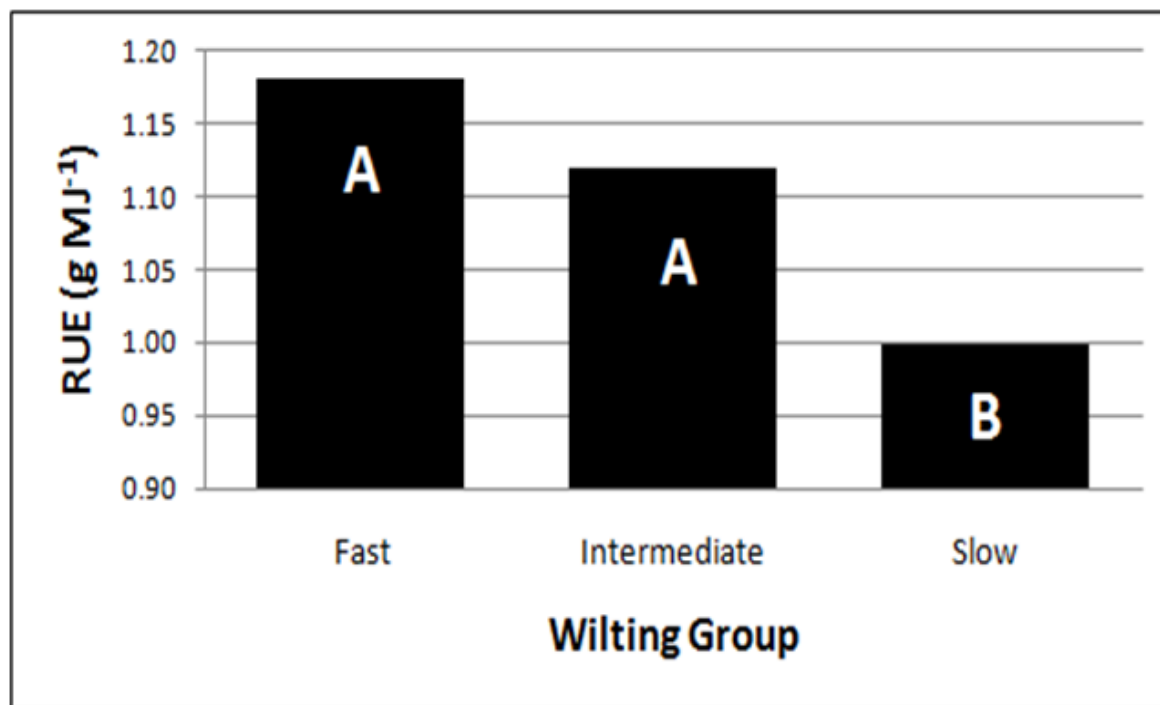
Ries, Purcell, Carter, Edwards, King. 2012. Crop Sci. 52:272-281.

Wilting vs. Radiation Use Efficiency (RUE)



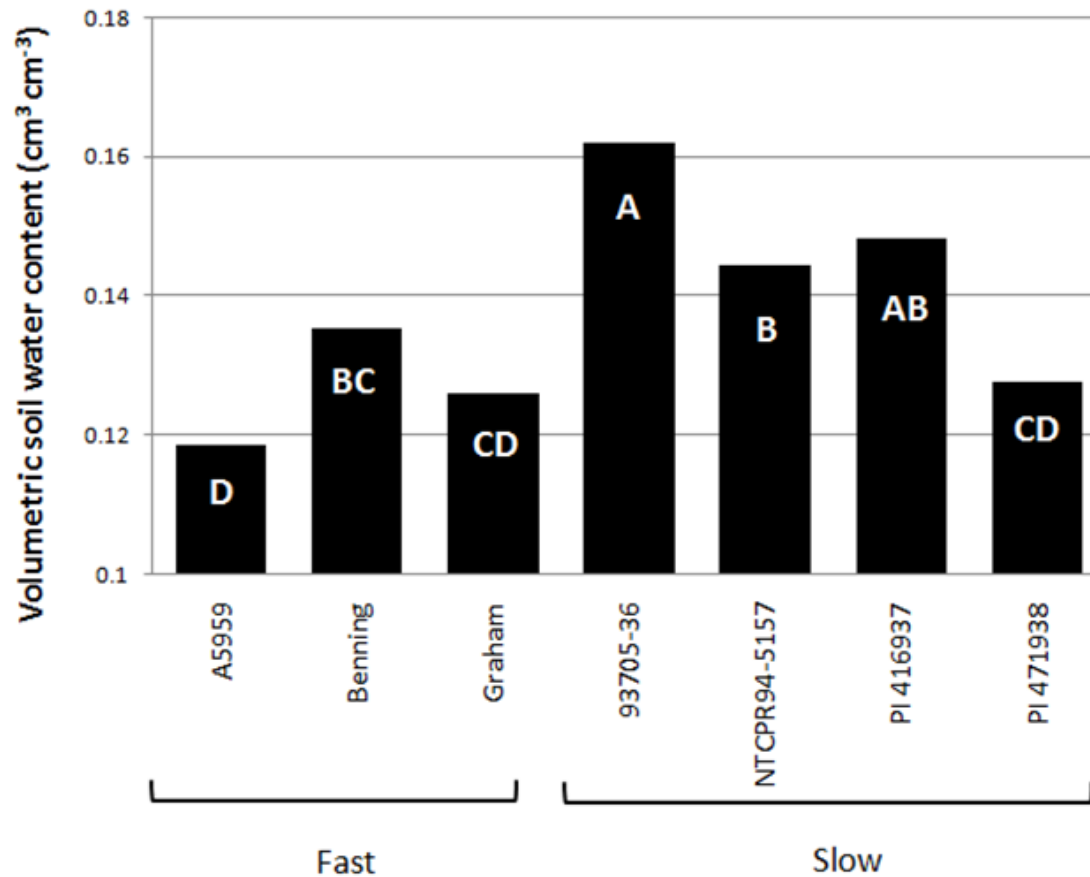
Ries, Purcell, Carter, Edwards, King. 2012. Crop Sci. 52:272-281

Source	DF	MS	F	Pr > F	R ²
Model	13	0.06	4.70	<0.0001	0.60
Error	40	0.01			
Total	53				
Wilting Group	2	0.18	13.04	<0.0001	
Genotype (Wilting Group)	6	0.03	2.15	0.07	



Ries, Purcell, Carter, Edwards, King. 2012. Crop Sci. 52:272-281

Wilting vs. Soil Water Content



Ries, Purcell, Carter, Edwards, King. 2012. Crop Sci. 52:272-281.

QTL Mapping & Yield Effect

- **Benning x PI 416937**

5 environments: AR (2007, 2009), NC (2009, 2010), and KS (2010)

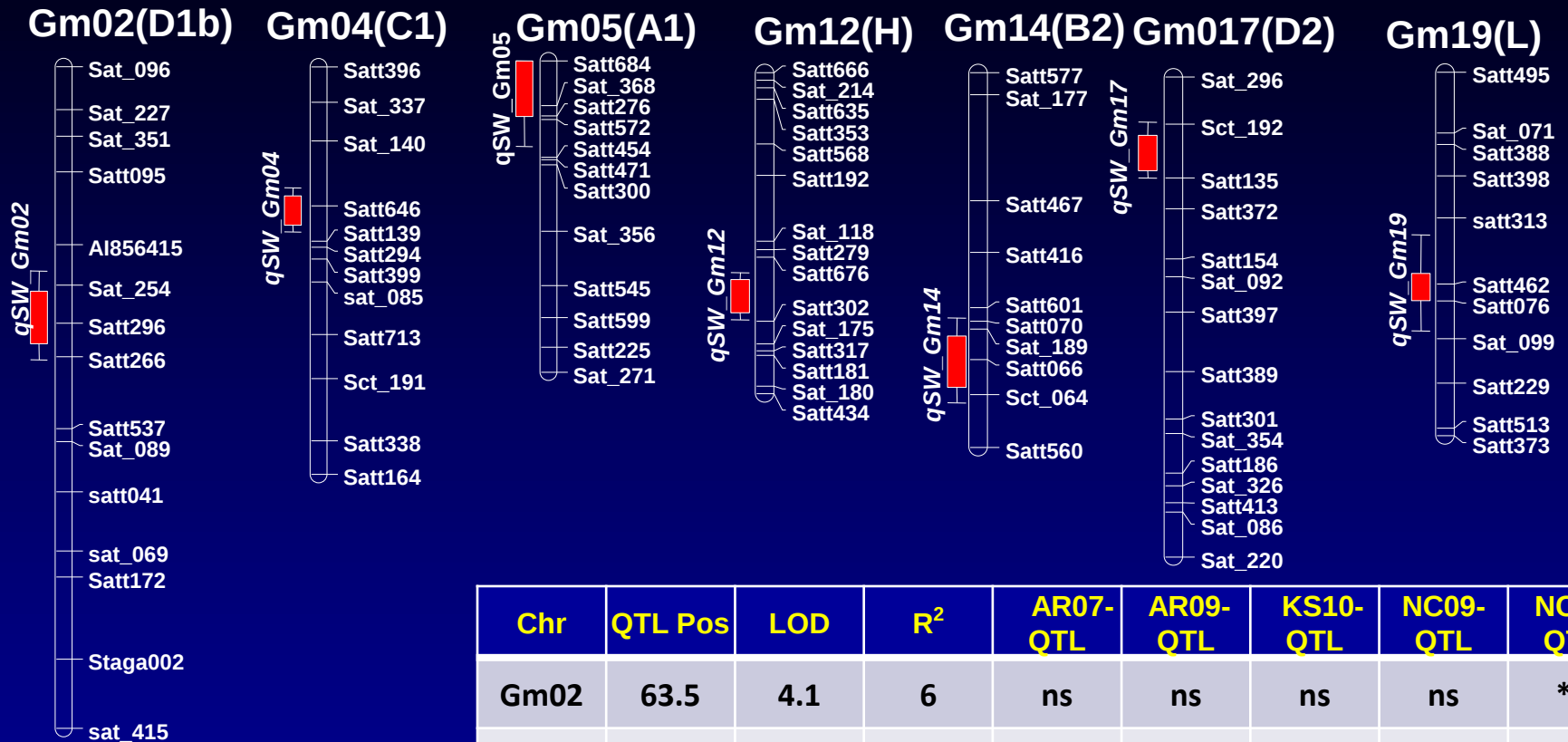
- **Hutcheson x PI 471938**

14 environments (2000, 2001)

9 irrigated & 5 non-irrigated



Canopy Wilting QTL Mapped in Benning x PI 416937



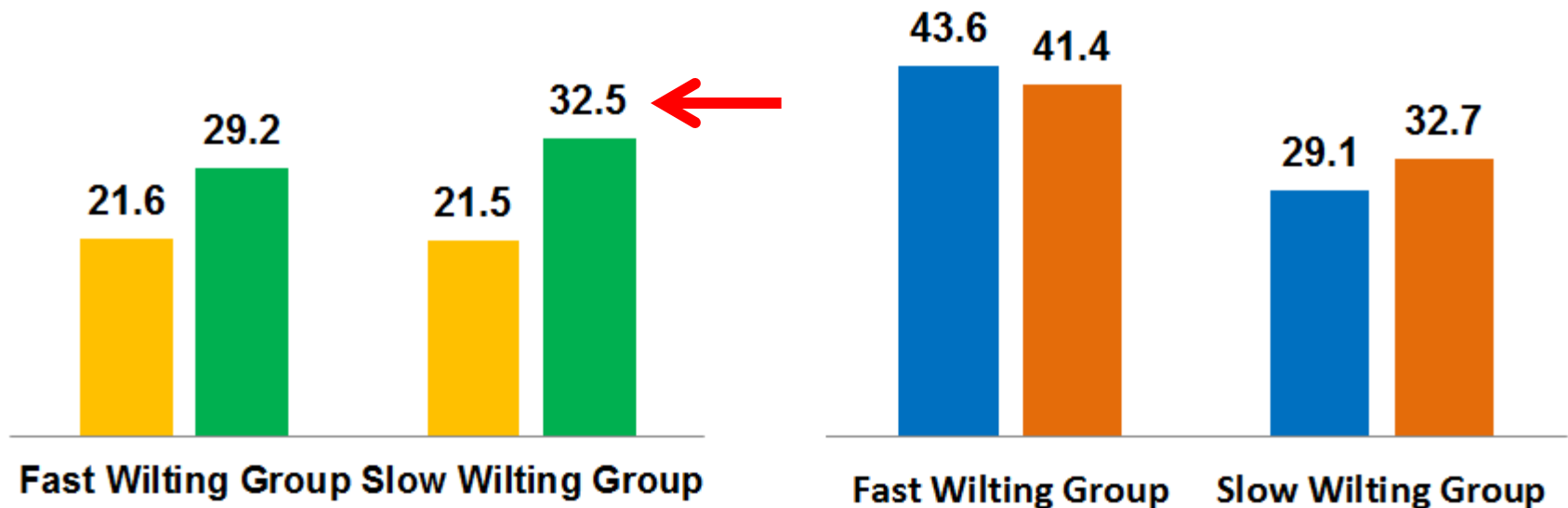
Chr	QTL Pos	LOD	R ²	AR07-QTL	AR09-QTL	KS10-QTL	NC09-QTL	NC10-QTL
Gm02	63.5	4.1	6	ns	ns	ns	ns	**
Gm04	36.9	7	9	**	ns	ns	ns	**
Gm05	8	2.6	4	**	ns	ns	**	ns
Gm12	56.8	9.1	27	**	**	**	**	**
Gm14	74.2	5.2	8	ns	ns	ns	ns	ns
Gm17	20.2	7.8	13	ns	ns	ns	**	**
Gm19	55.7	3.2	8	ns	**	**	ns	ns

Abdel-Haleem et al, 2012
TAG 125:837 - 46

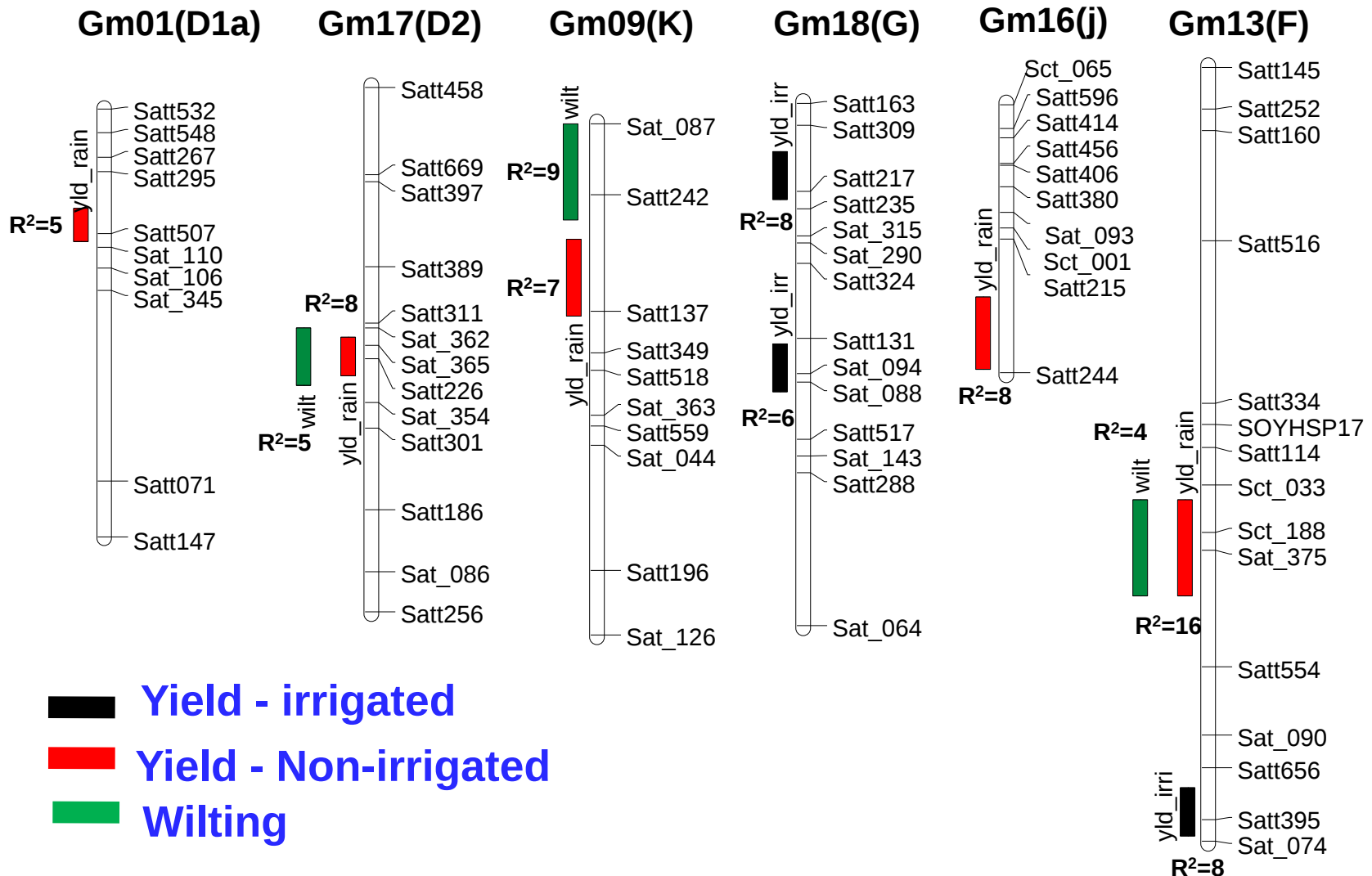
Wilting QTL Effect on Yield

- 27 RILs selected from Benning x PI416937 based on canopy wilting
- No yield difference between two groups in 2011
- 3.3 bu/a yield advantage in slow wilting group in 2012

■ 2011 Athens Yield (Bu/a) ■ 2012 Athens Yield (Bu/a) ■ Wilting Score (5 Env) ■ Wilting Score (KS, 2012)



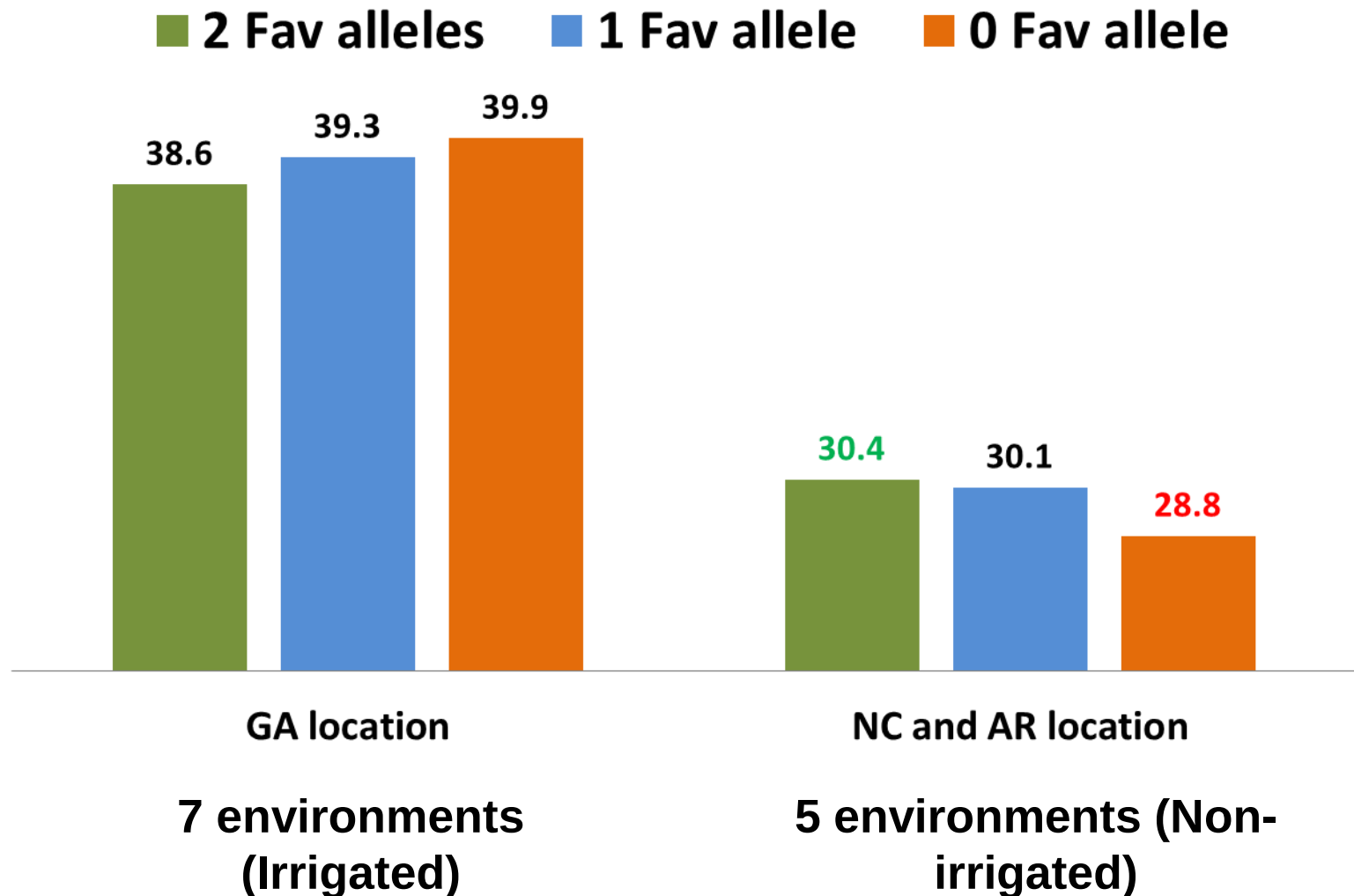
Wilting & Yield QTL in Hutcheson x PI471938 Population



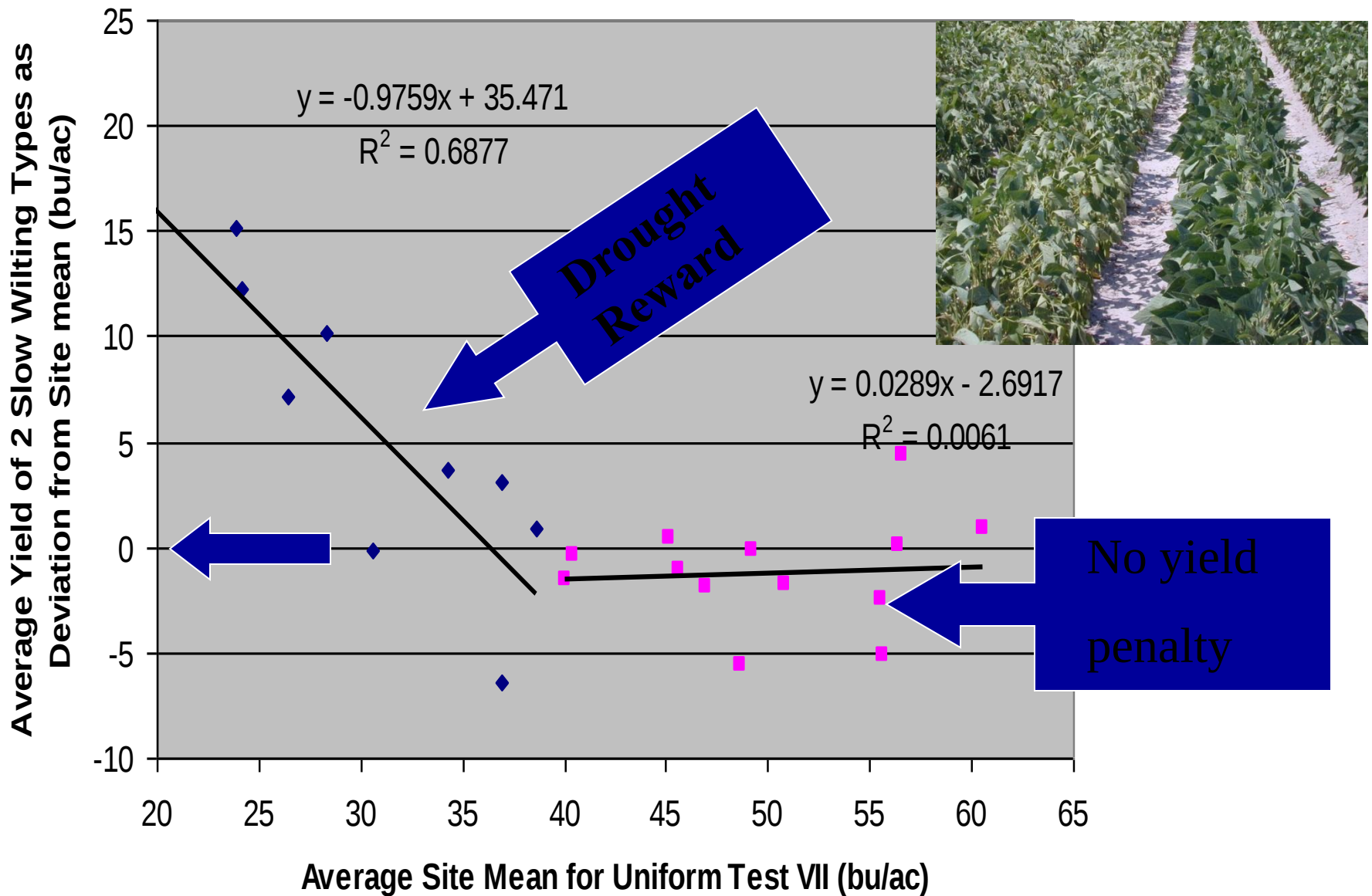
Confirmation of Yield QTL from PI471938 Under Irrigated/Non-Irrigated Condition

- NIL Development
 - Developed NILs using Heterogeneous Inbred Families (HIF)
 - Heterogeneous lines at QTL regions were selfed
 - Lines homozygous for PI 471938 and Hutcheson alleles were selected for testing
- Yield tests
 - NILs were tested at 12 environments (2008 – 2011)
 - AR (2009 & 2010) and NC (2008, 2009 & 2010) - irrigated
 - GA (2008, 2009, 2010 & 2011) - non-irrigated
 - Yield data were collected from replicated plots

Wilting QTL from PI471938 on Yield



Yield of Slow Wilting Types in the USDA Uniform Test 2004-06



New ERA Slow Wilter in Regional Trials



MG 8	Ent.	Hi Yield Env.	Lo Yield Env.
		> 55	< 55
		BU/ AC	
NEW	N05-7432	59	45
CHECK	N8001	58	41
	Advantage	0	4
25 Loc		5	20

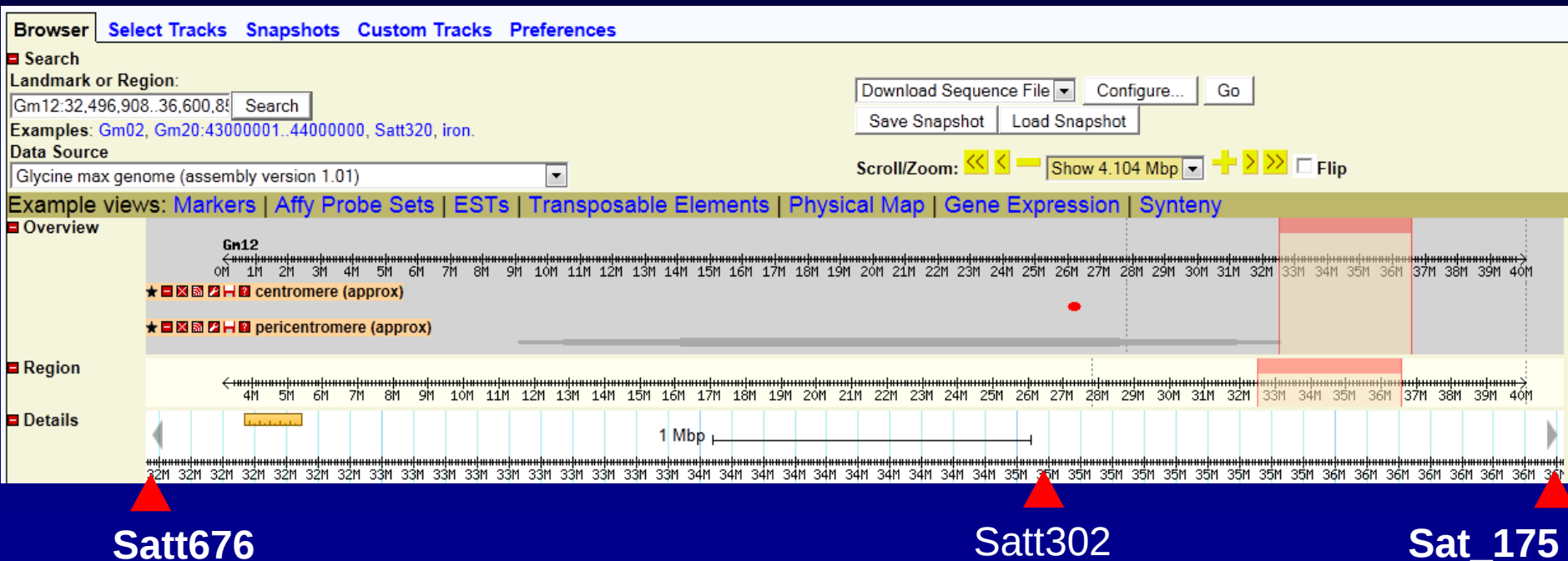
37% PI



New Drought Line
N05-7432
Top Yields

Development of SNP Markers Using Infinium Chips for MABC

Glycine max genome: 4.01 Mbp between Satt676 and Sat_175 on Gm12



- 349 SNPs between Satt676 and Sat_175 from Infinium chip data
- 80 SNPs were polymorphic between two parental lines, Benning and PI416937
- SNP marker development for high-throughput genotyping

Converting Infinium Chip SNPs for Genotyping

Sequence

Parent 1

ACAAAGATTCGGCTAGGCGCGA**A**TCGACGCGGGCGAAGAACGTGCTTCAAA

Parent 2

ACAAAGATTCGGCTAGGCGCGA**T**TCGACGCGGGCGAAGAACGTGCTTCAAA

Allele specific Primer design

FAM tail **A**-specific primer



VIC tail **T**-specific primer



Common reverse



PCR and fluorescent detection

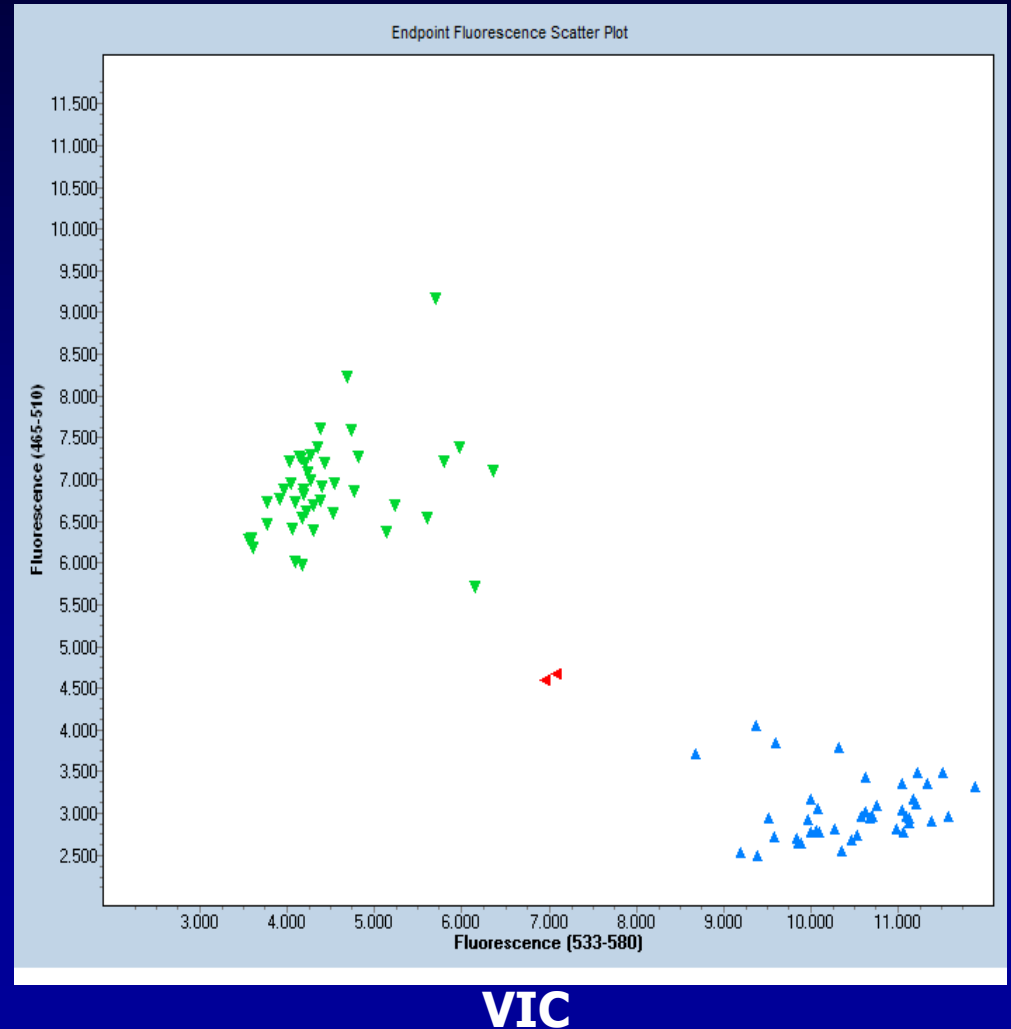


KASP SNP Assays for MABC and MAS

SNP marker developed
from Gm12_34289684_C_T

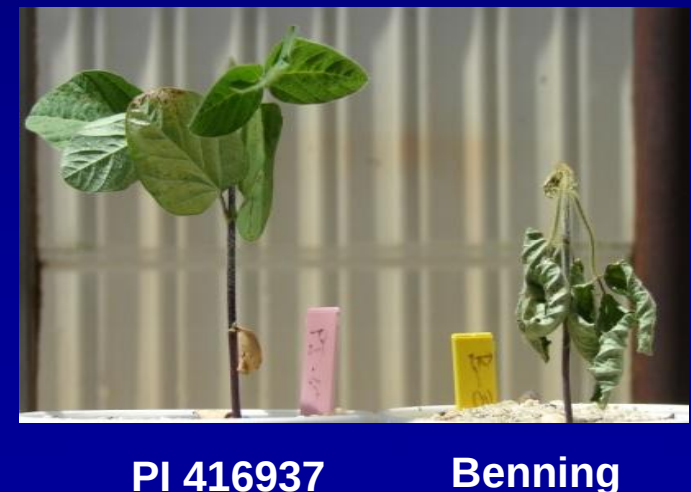
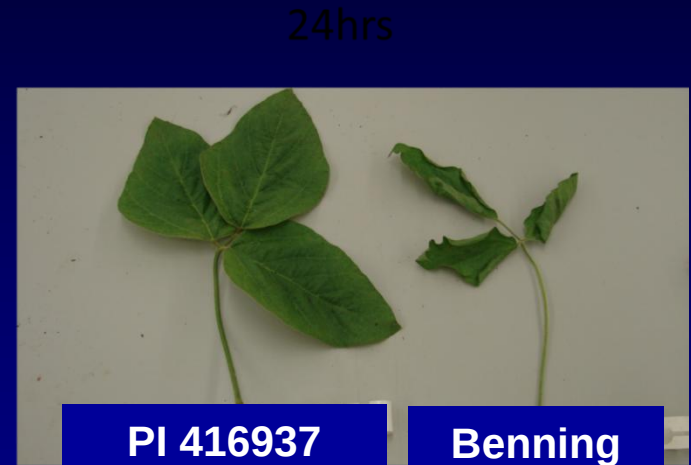
- Benning CC (FAM)
- PI416937 TT (VIC)

FAM



Validation and Fine Mapping of Canopy Wilting QTL from PI416937 using RNAseq

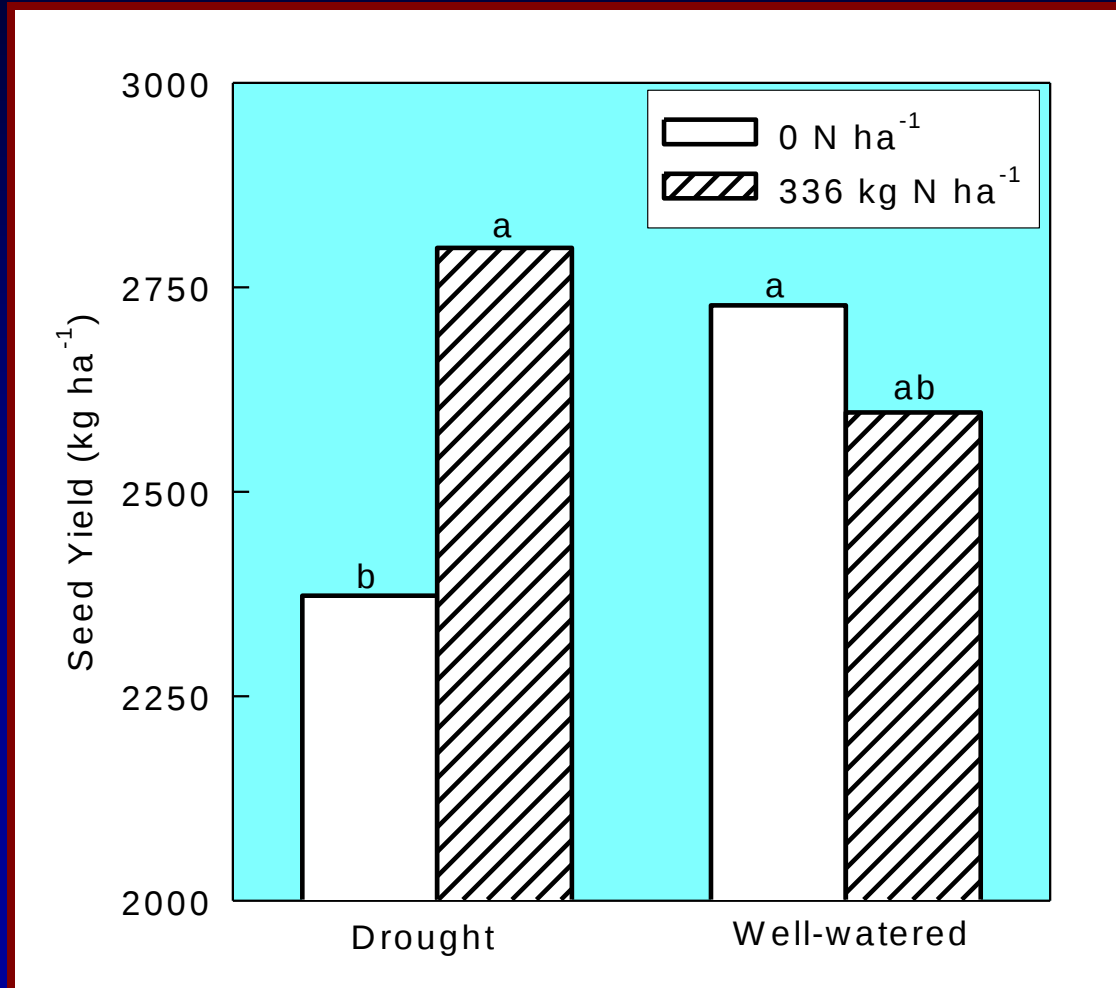
- Tissue collected from PI416937 and Benning under stress condition
- Investigate the genes overexpressed/under expressed at QTL regions
- Investigate the genes overexpressed/under expressed at a whole genome level



Prolonged Nitrogen Fixation Under Drought

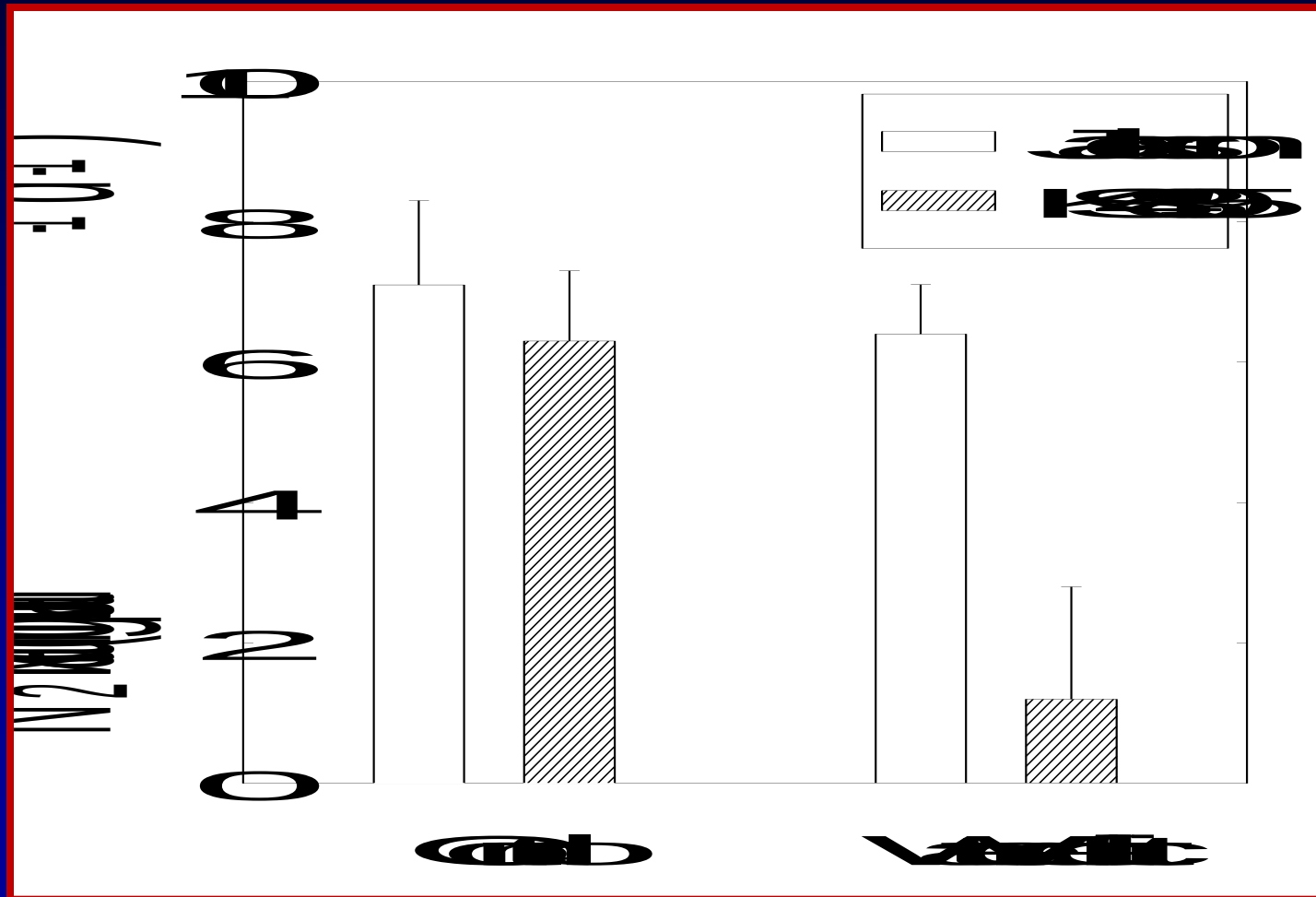


Nitrogen vs. Yield Under Drought



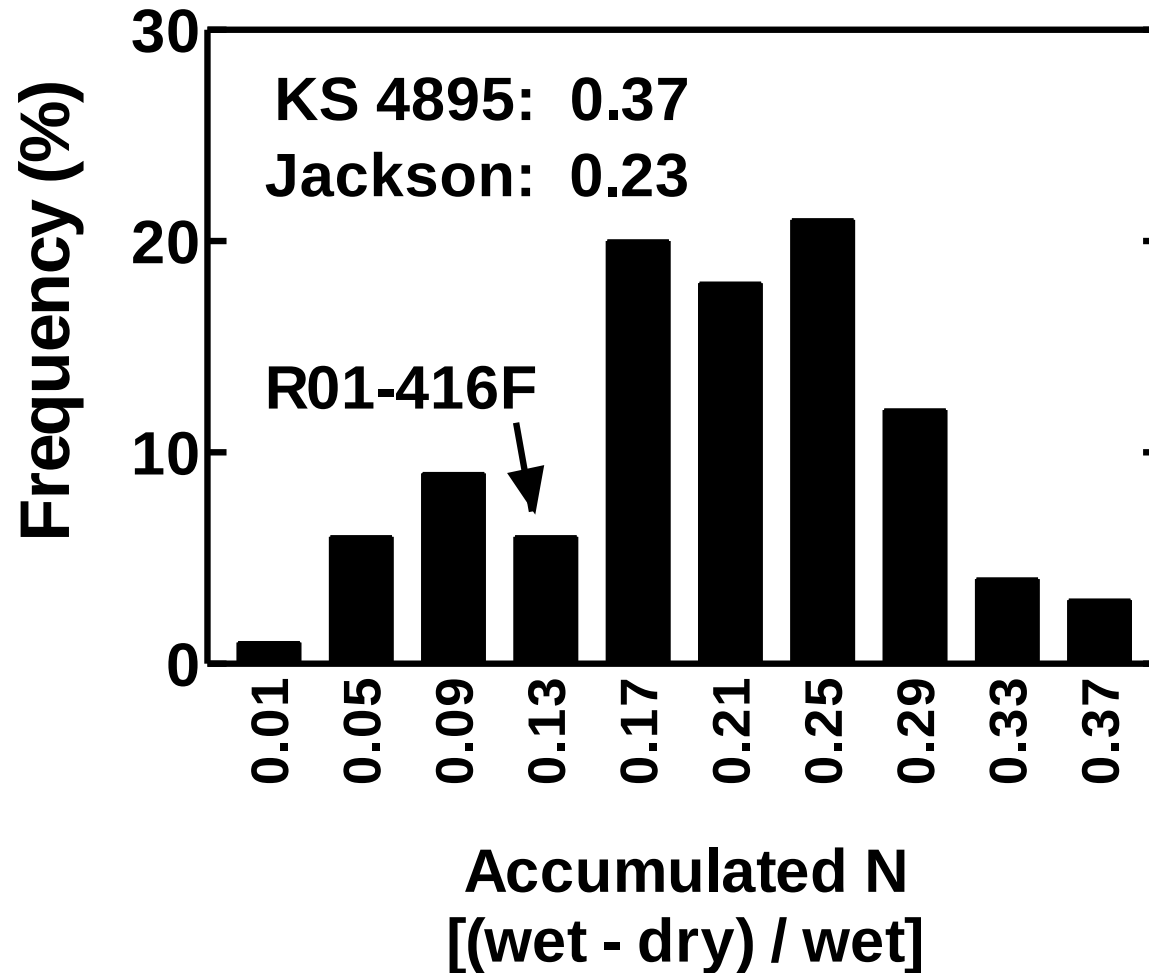
Purcell and King, 1996. J Plant Nutr 19:969-993

Genotypic Difference in N₂ Fixation Under Drought

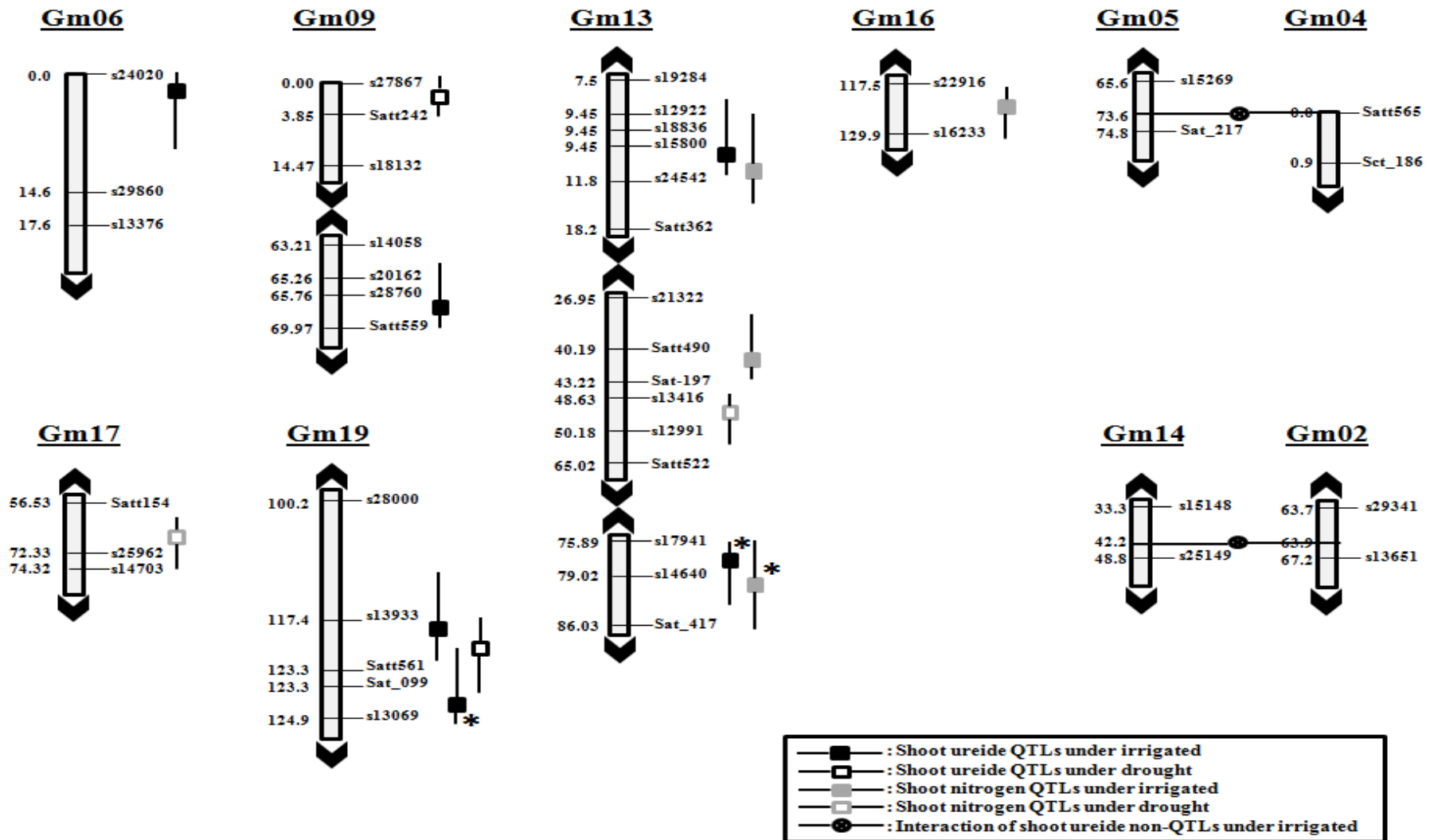


Purcell et al, 1997. Plant Soil. 196:101

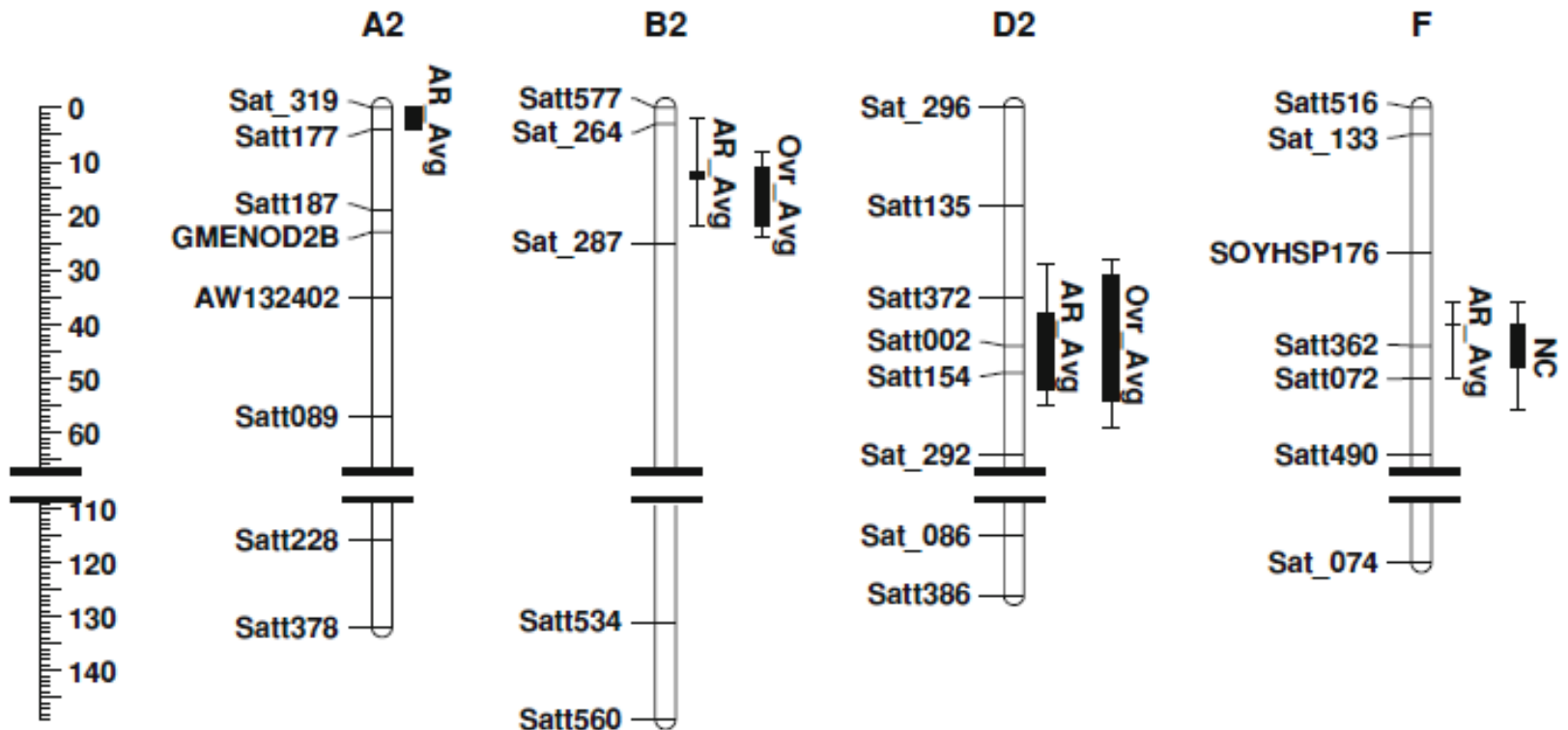
N2 Distribution in Jackson x KS 4895 Population Under Drought



N2 Fixation QTL Under Drought

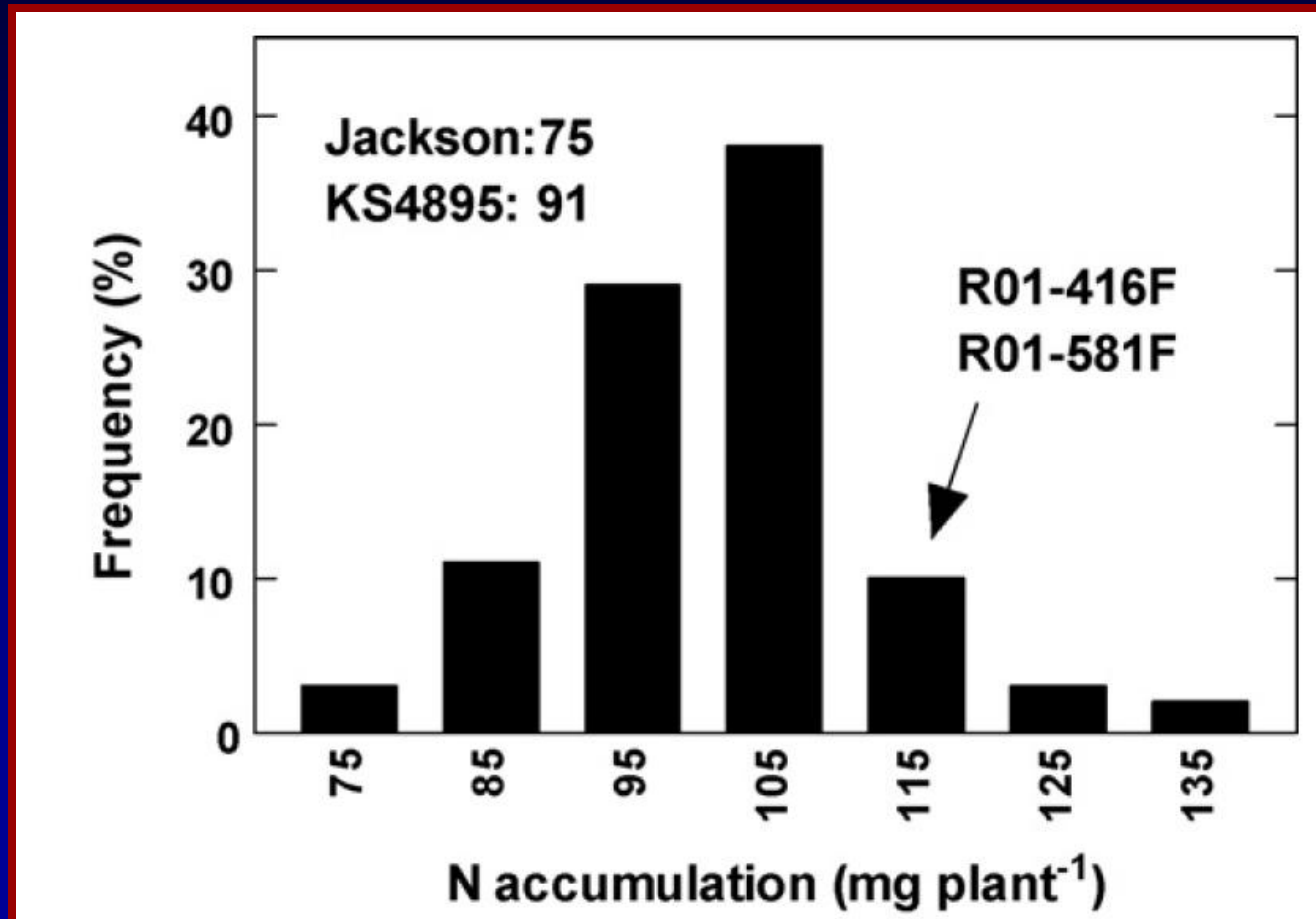


Slow Wilting QTL in the Same N2 Fixation Population



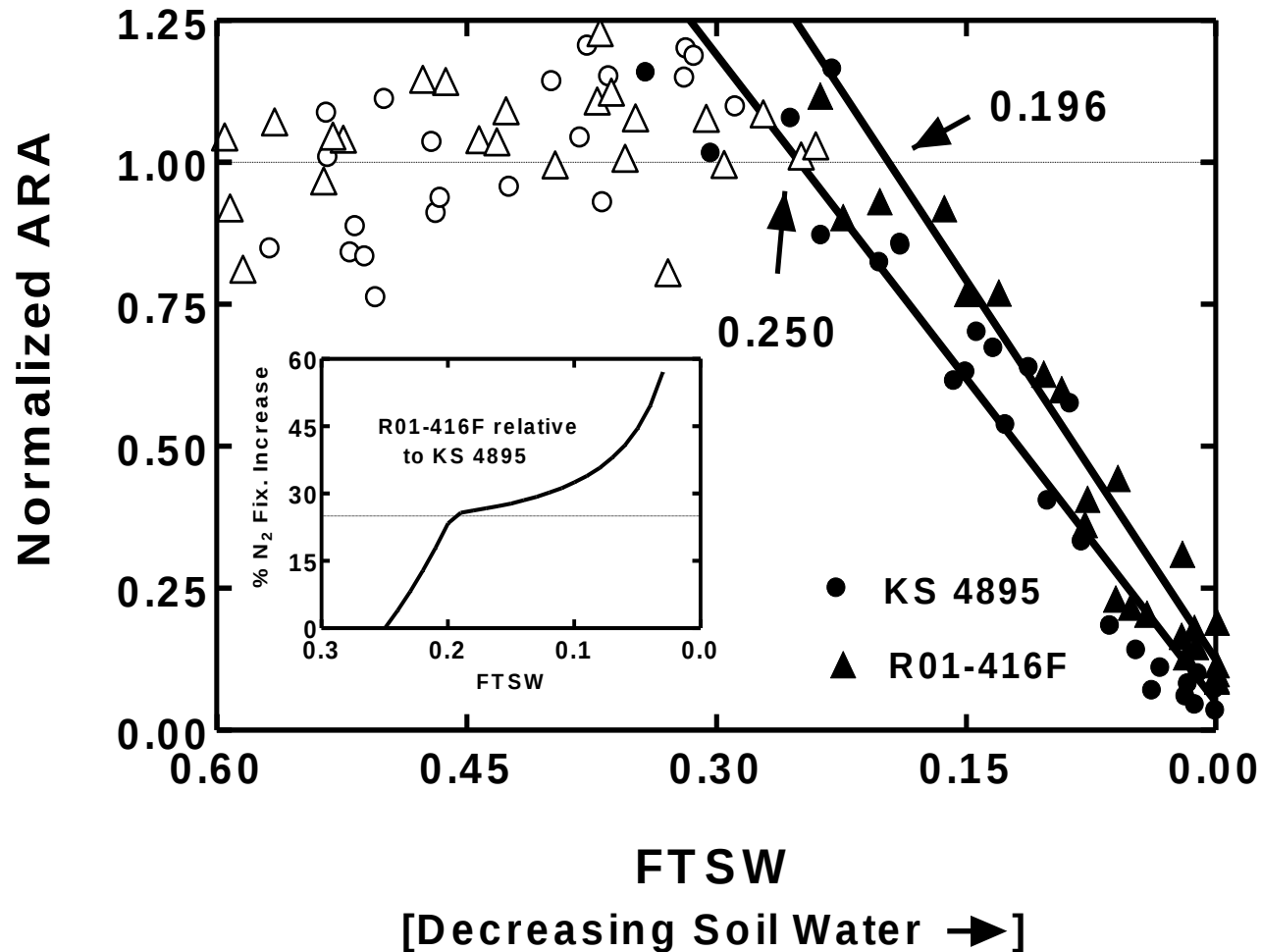
Charlson et al. (2009) Theor. Appl. Genetic. 119:587-594.

N₂ Fixation vs. Yield Under Drought

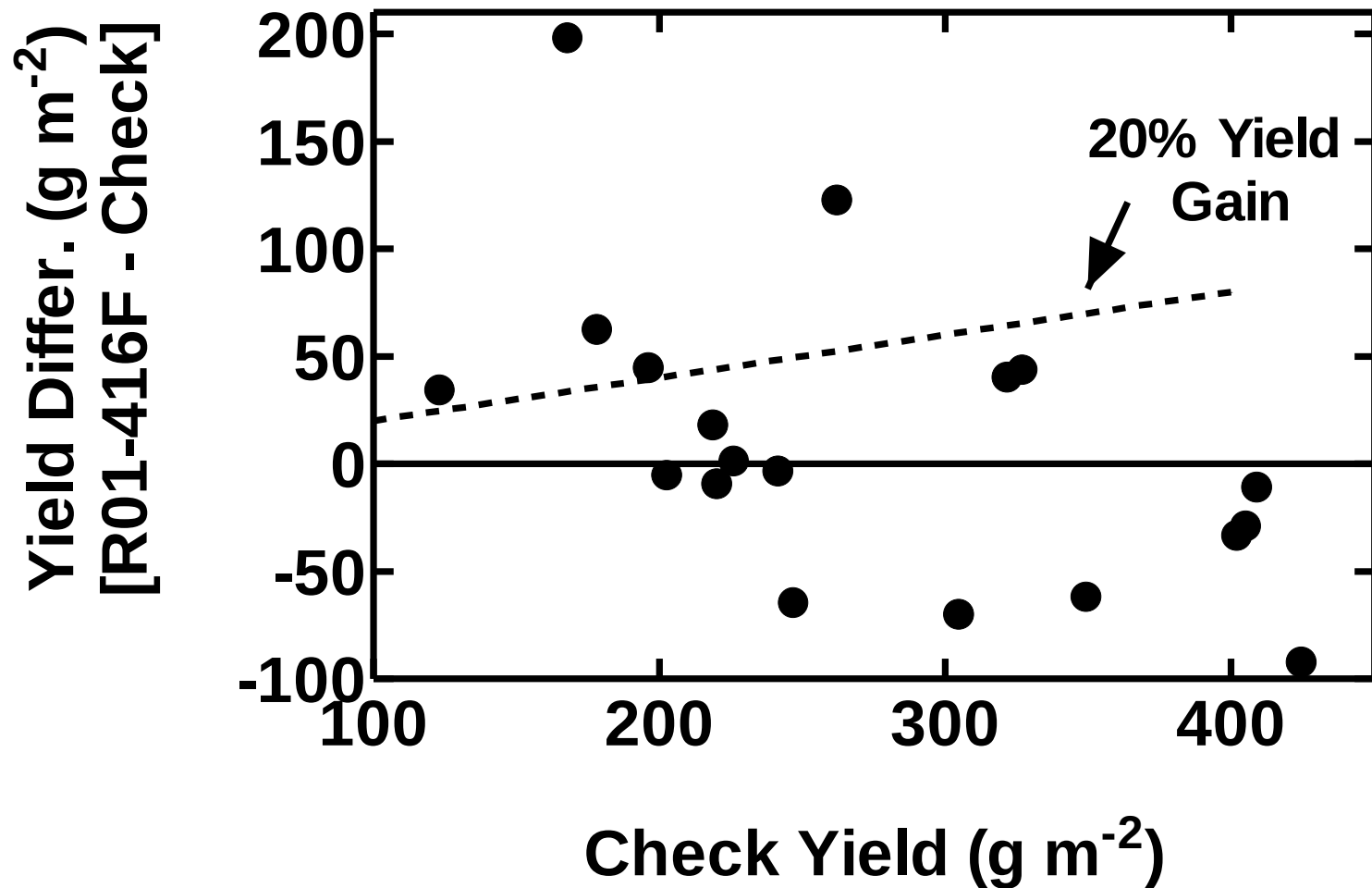


Sinclair et al, 2007. Field Crops Res 101:68-71

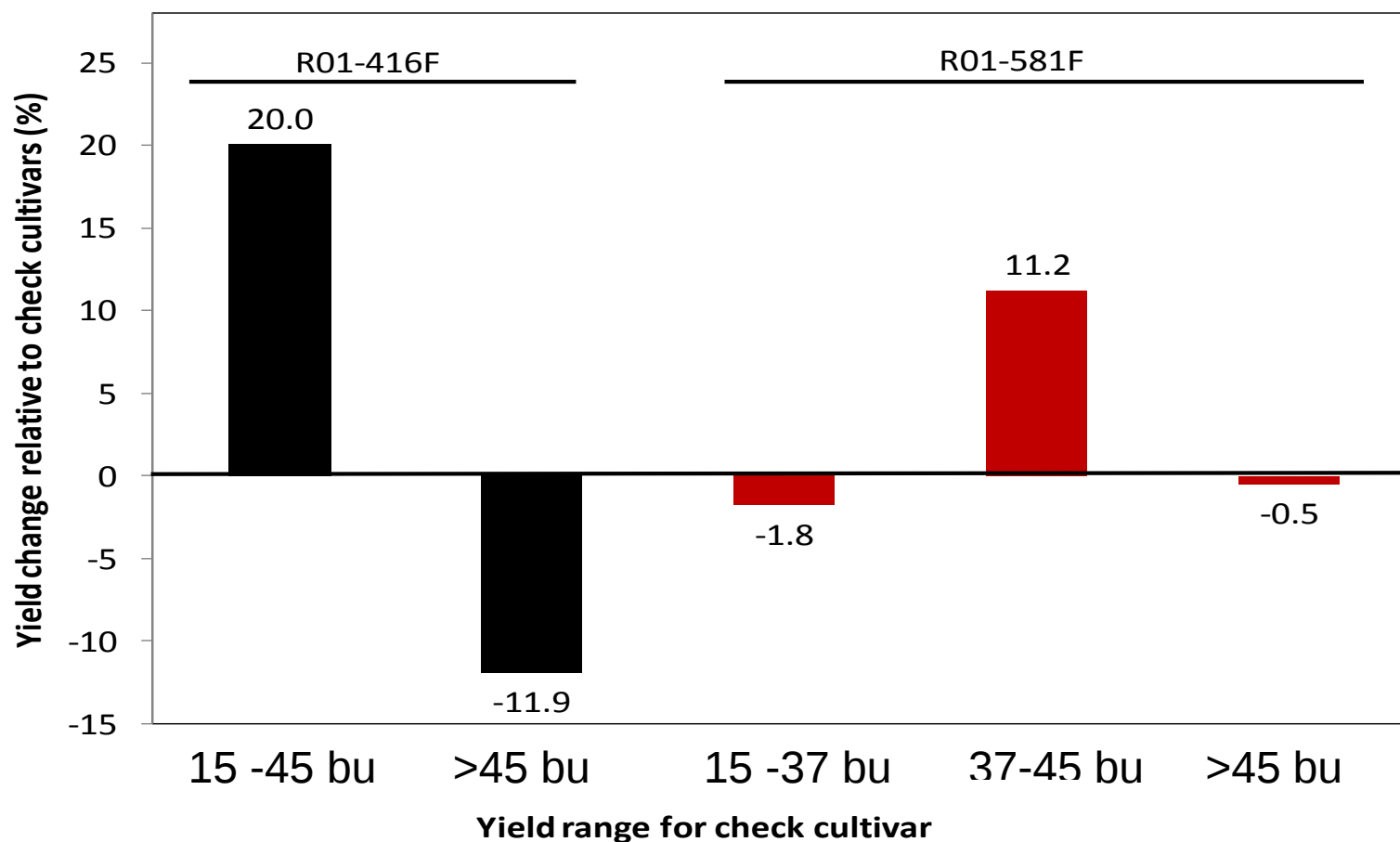
Prolonged N₂ Fixation Under Drought



Yield Advantage with N₂ Fixation Under Drought



Yield Advantage with N2 Fixation Under Drought



Sinclair, Purcell, King, Sneller, Chen, Vadez. 2007. Field Crops Res 101:68-71

Soybean Root Characteristics

$$\text{Yield} = \text{WU} \times \text{WUE} \times \text{HI}$$

Using Available Water

Gaining access to more water through improved root architecture



Root Characteristics

- Deep rooting
- Fast rooting
- Fibrous rooting



“Root Race”

- 40 trenches: 1.2 m deep x 30 m long
- drip irrigation line in each trench
- permanently marked trench location
- injection of herbicide
- rating of shoot symptoms





Root Race

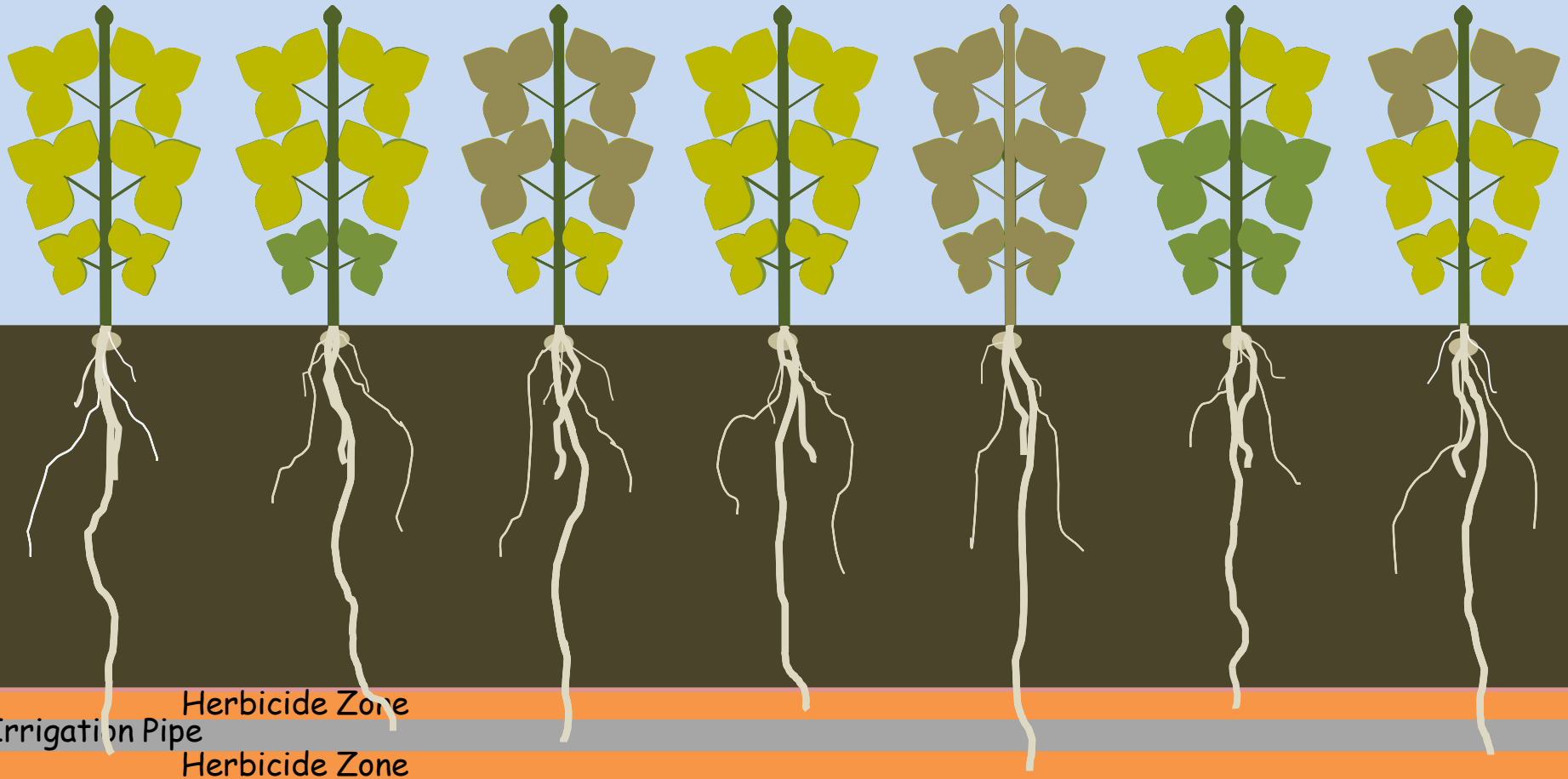


Observation 1

Observation 2

Observation 3

Observation 4





Using Available Water



Root Race Observations – Early





Root Race Observations – Late





Using Available Water



Slanted Tubes

depth, elongation rate, complexity

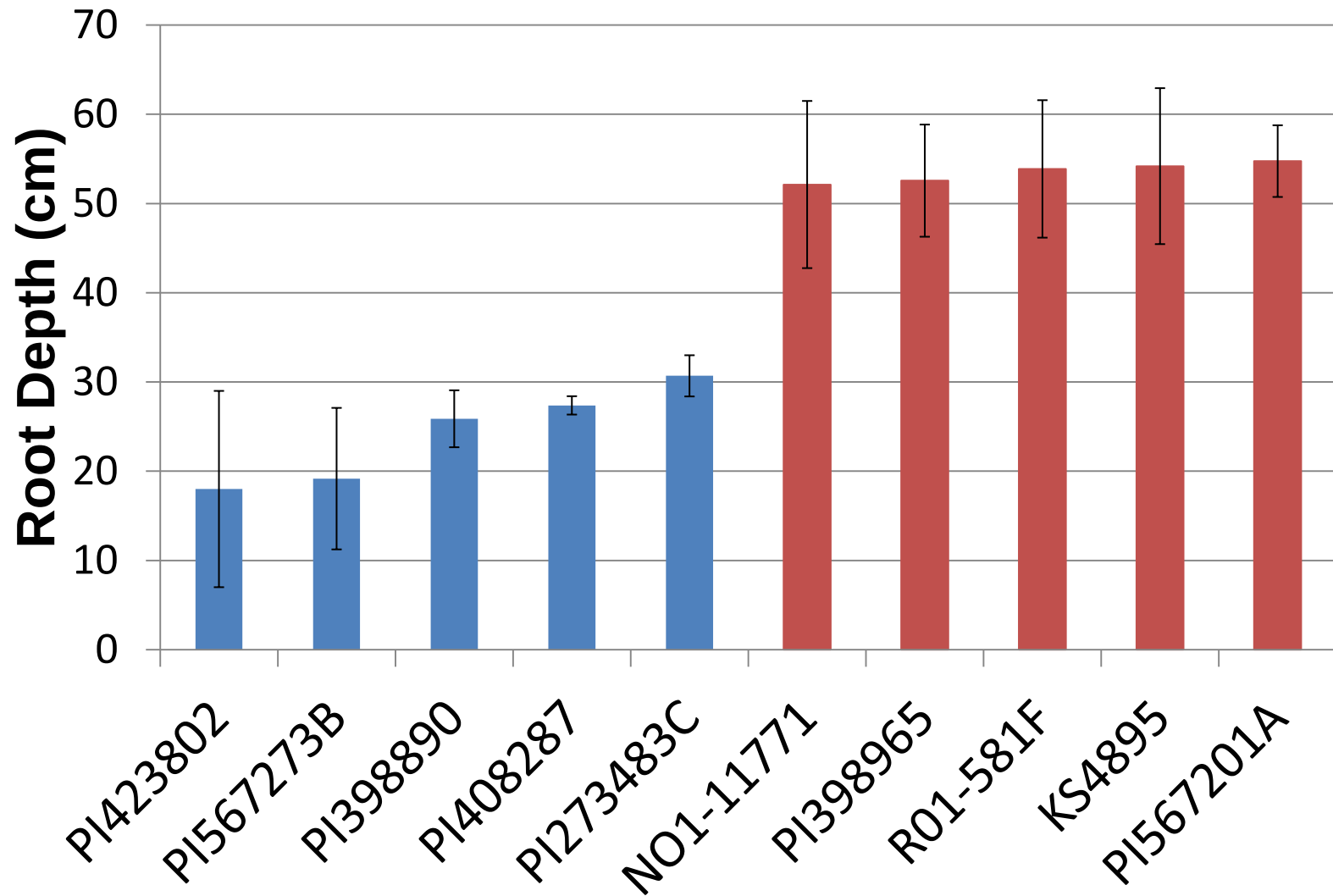




Using Available Water



GH Confirmation for Rooting Depth



Soybean Root Characteristics

Summary

- > 800 lines screened in the field
- > 200 lines re-examined in the greenhouse
- Characterization of 16 lines by soil coring and soil moisture depletion
- Development of RIL populations – F3
- Development of methodology for phenotyping of populations

QTL for Fibrous Root

Benning x PI 416937

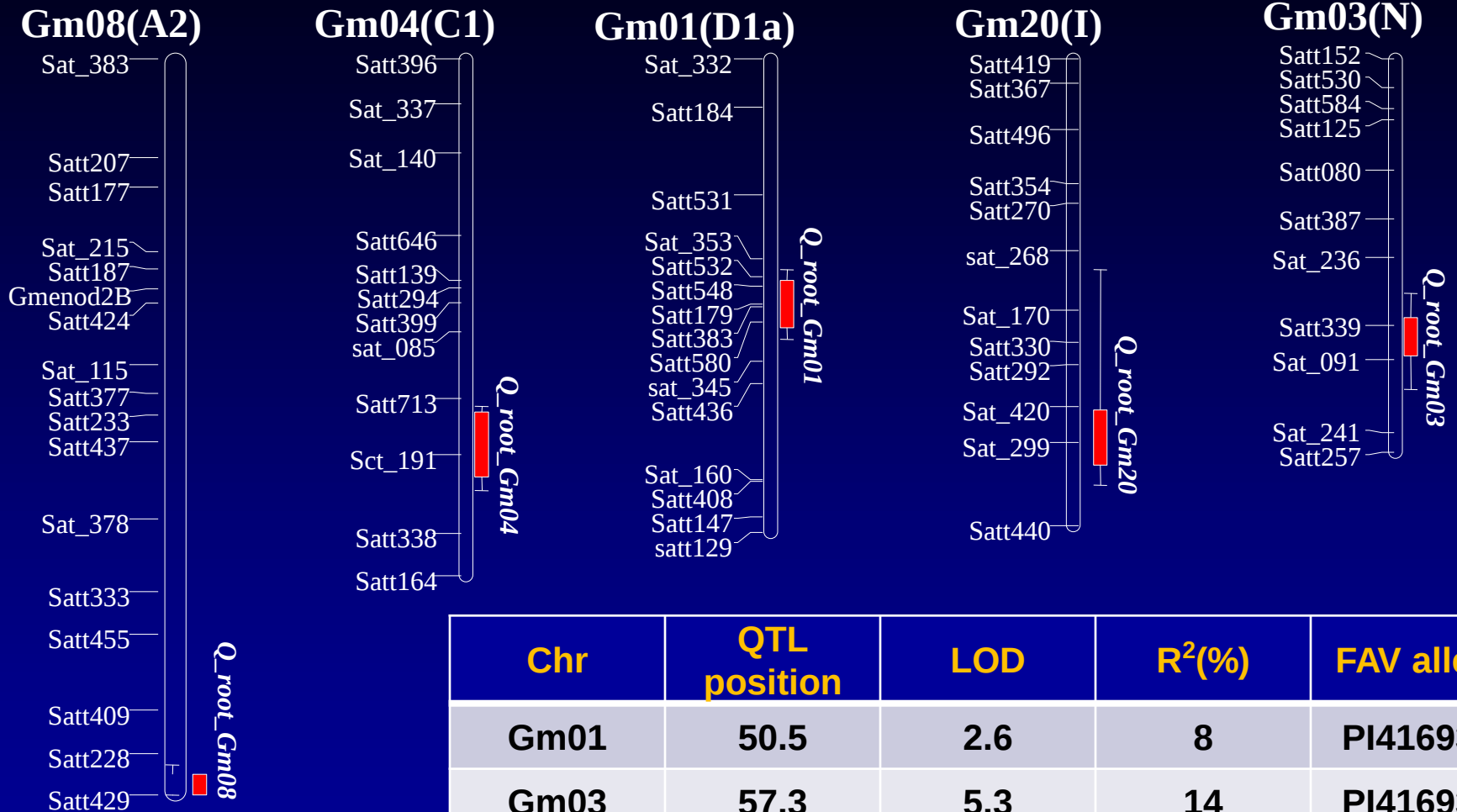
- Hill plots
- 2 locations
- Plots inverted at R5 using a peanut inverter
- Roots visually rated on the scale 1 (course roots) to 8 (high fibrous roots)



Peanut Inverter for Root Phenotyping

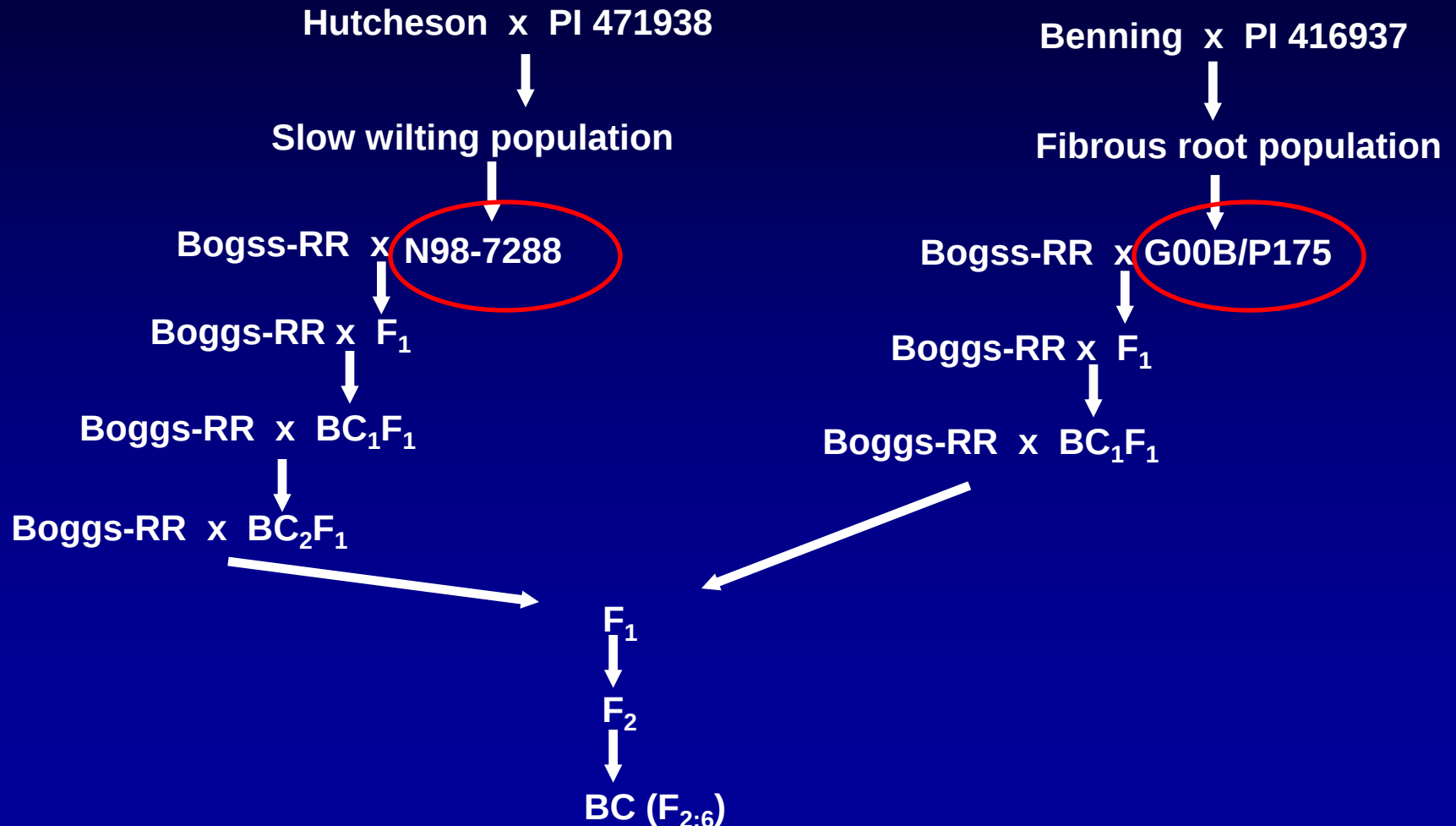


Fibrous Root QTL Mapped in Benning x PI 416937



Abdel-Haleem et al, 2010
TAG 122:935-946

Pyramiding Slow Wilting and Fibrous Root QTL from PI 471938 and PI 416937



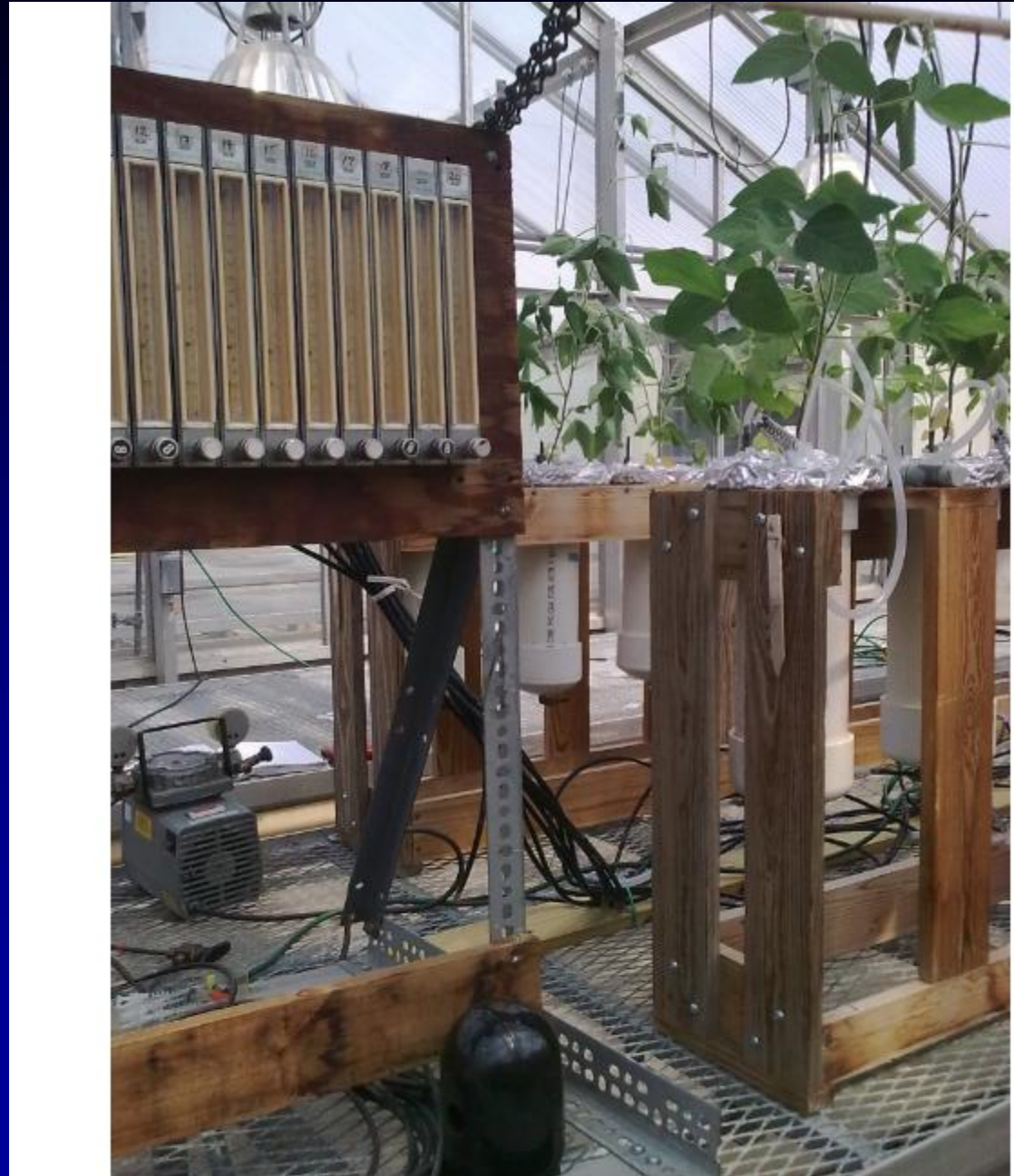
Backcross Slow Wilting and Fibrous Root QTL from PI416937 into Elite Lines using MABC

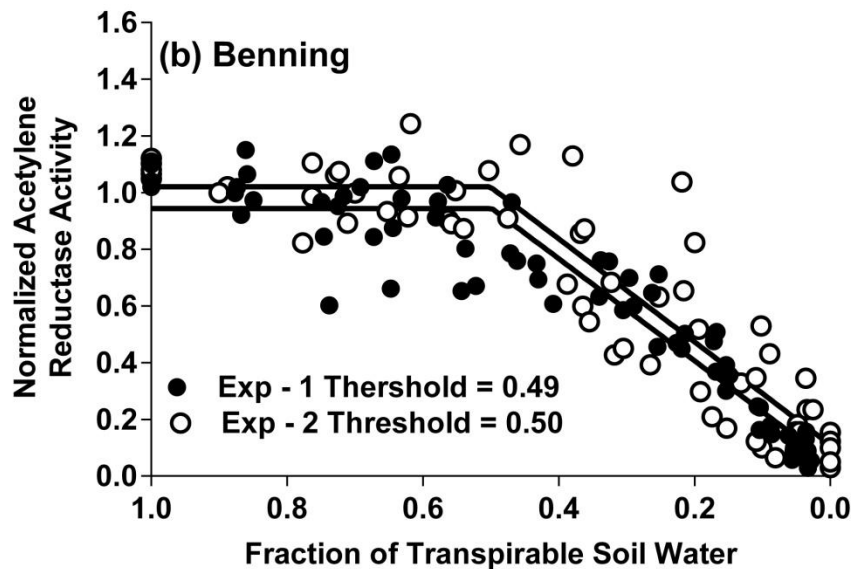
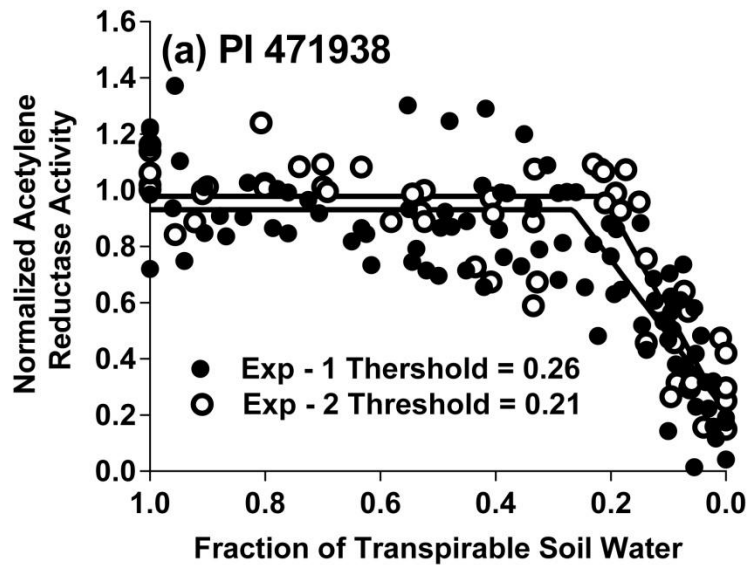
- Elite Lines
 - G00-3213
 - G00-3880
 - NE3001
- QTL
 - 6 slow wilting QTL
 - 4 fibrous root QTL



Acetylene Reduction As an index of N₂ fix

PI 471938





A New Source of Prolonged N₂ Fixation Under Drought in the Slow Wilting PI 471938

Drip Irrigation



Nebraska

Before vs. After Irrigation



Drought vs. Flood

Not enough water ?

Too much water ??

Related mechanisms ???



Field Flood Test



Flood Damage



Flood Tolerant Lines

UA 4805, Ozark, Osage, Anand, Manokin

NC-Roy, Young, Boggs-RR, Narrow

RA-452, PI 471931, PI 471938



Also Drought Tolerant

Drought Tolerance

- **A complex Trait !!!**
- **Research Continues...**