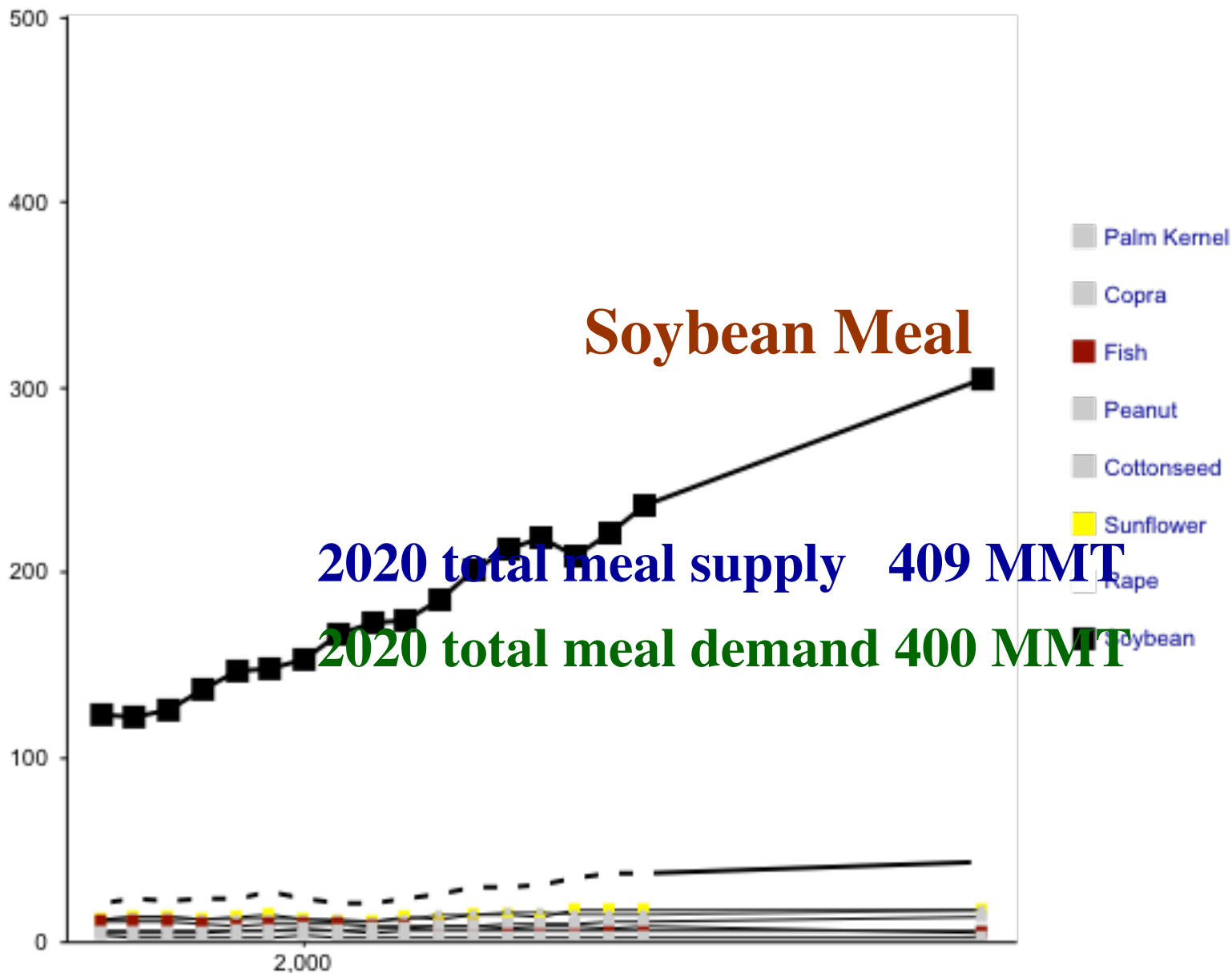


Genetic Variation and Quantification of Amino Acids in Soybean

Vince Pantalone

University of Tennessee







Soybean Meal

Highly Desirable Feed Ingredient

- **Comparative advantages**
 - **availability**
 - **crude protein**
 - **lower fiber**
 - **greater digestibility**
 - **higher net-energy**
 - **favorable levels growth-limiting amino acids**

(Wilson, 2012)



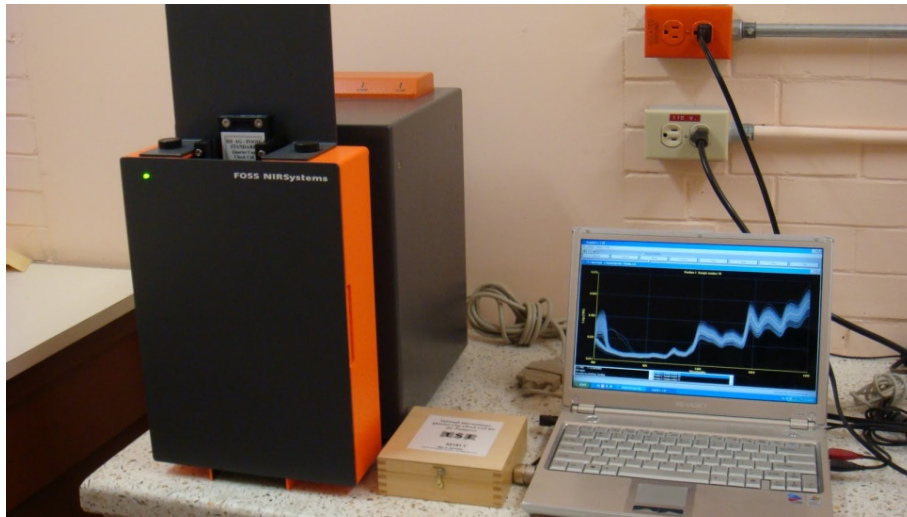
Soybean Meal

- Still a **cost effective** source of amino acids
- Excellent protein source to mix with corn meal
- **Overconsumption** of AA can lead to **carbon dioxide** and **urea** production
- **Inadequate** quantities of AA can lead to **protein degradation**

Soybean Meal

- Different AA are important to different animals
- Met, Cys, Lys, Thr, and Trp for swine
- Met, Cys, Lys, Thr, Arg, and Val for poultry
- Soy is deficient in S-containing amino acids Met and Cys





FOSS 6500 NIRS

**Amino acid wet chemistry
(HPLC)**

Accurate

Costly

Time consuming

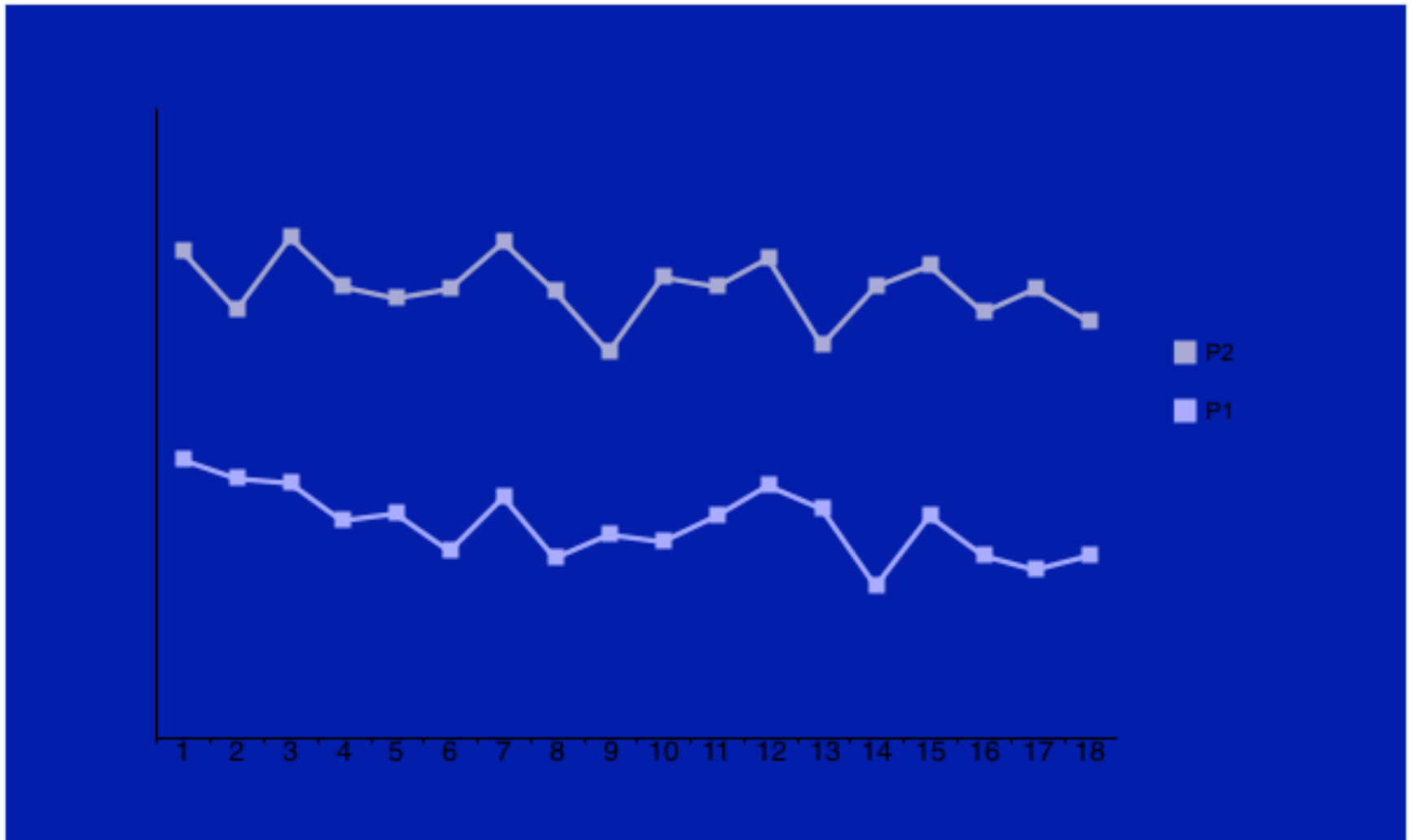


Perten DA 7250 NIRS

Amino acid NIRS

- Accurate only to calibration curve and sample number & distribution
- Easily affordable
- Swift – except seed grinding necessary

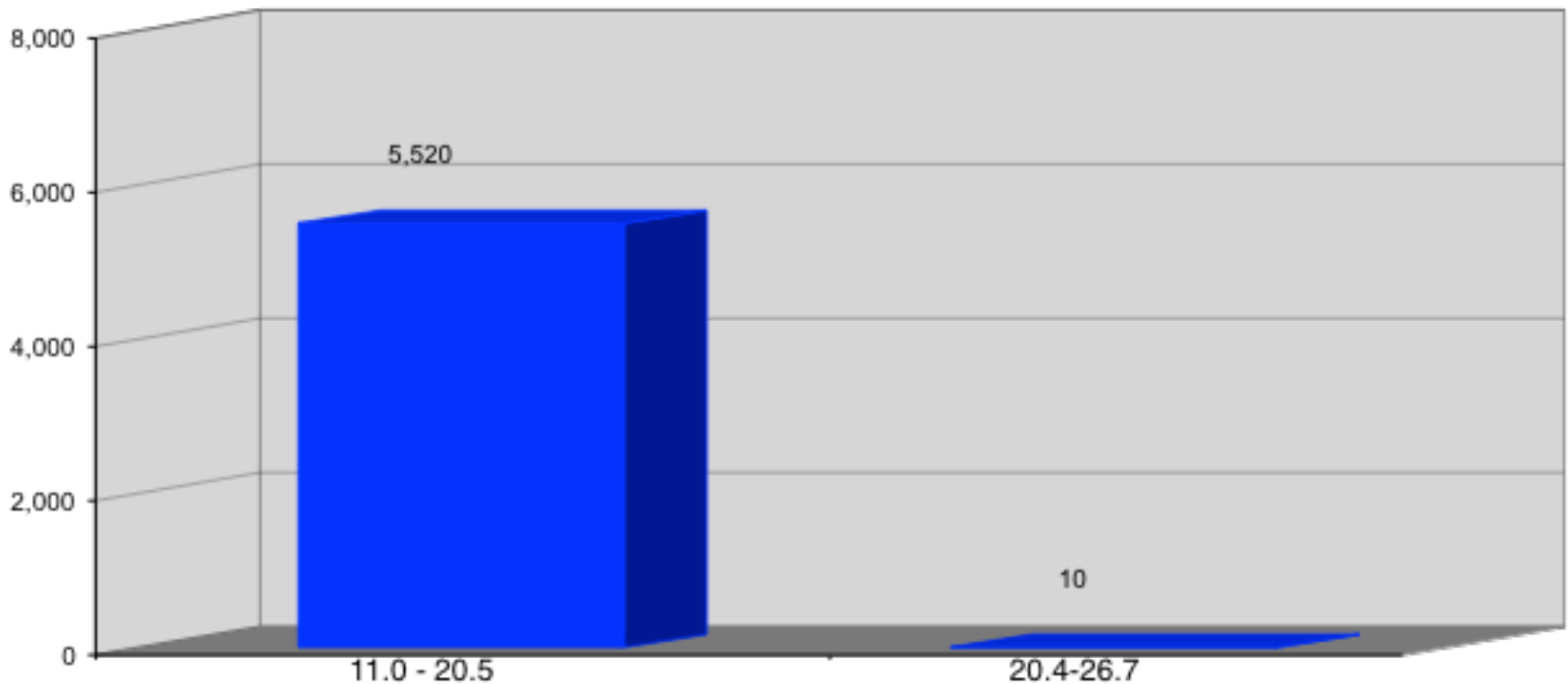
Met



**Prolina vs Brim parental lines at NCSU (Carlson; Cardinal)
18 field reps of parents showed P < 0.0001 genotypic differences for
Methionine concentration**

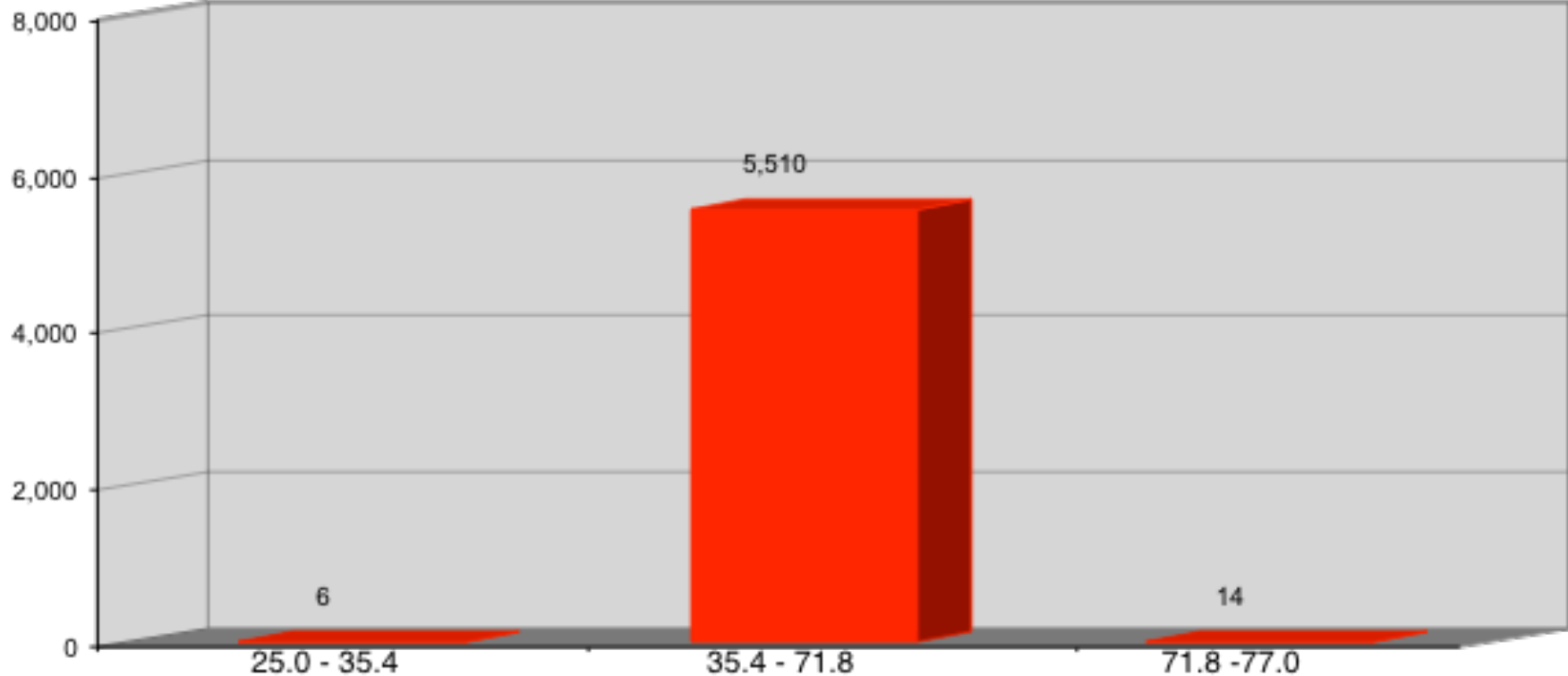
5,530 GRIN Soybean Accessions (g **CYS** / kg CP), FOSS 6500 NIR

No. of
Lines

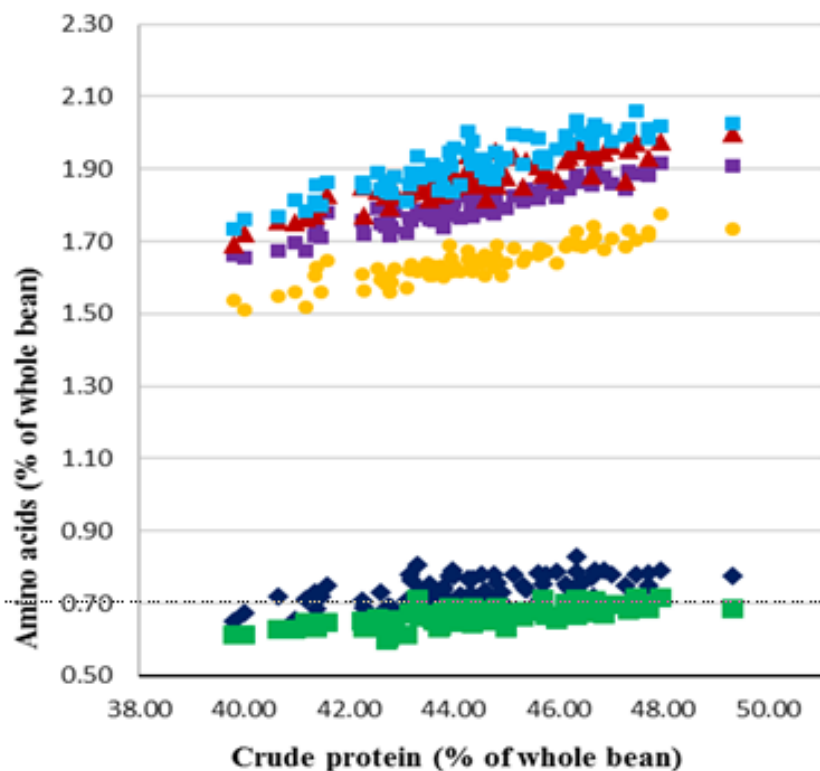


5,530 GRIN Soybean Accessions (g **LYS** / kg CP) FOSS 6500 NIR

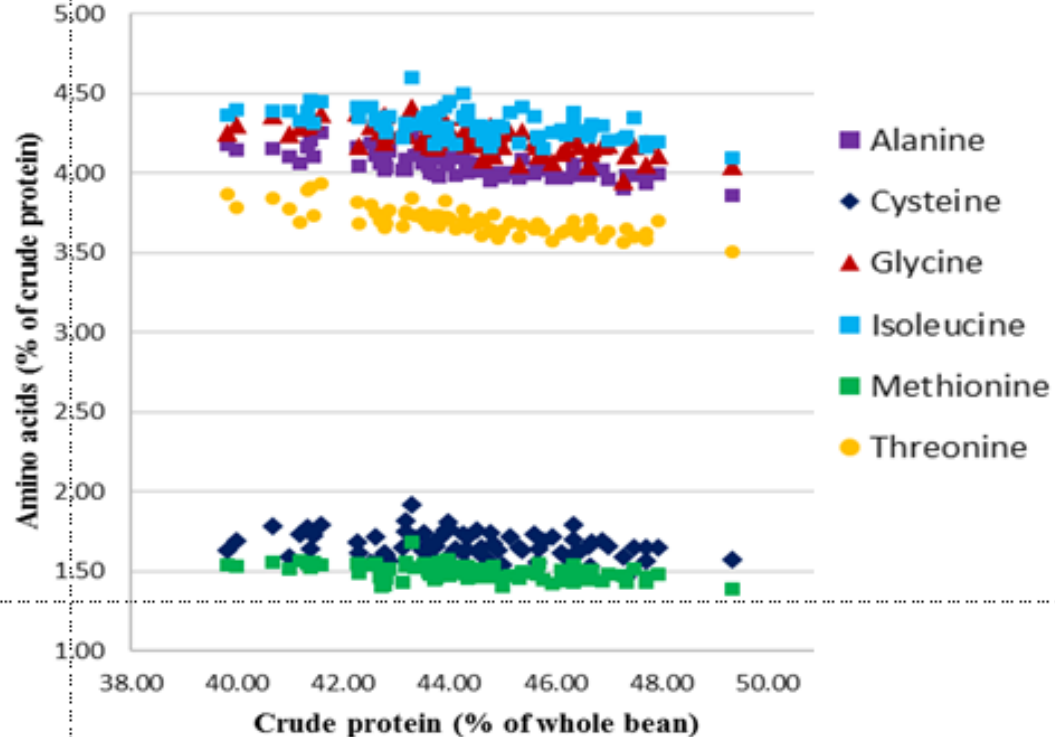
No. of
Lines



a)



b)



Glycine soja – core collection

As protein increases, the concentration of amino acids per kg crude protein decreases.

But slope is less than in *Glycine max*

Some amino acids e.g CYS show wider distribution, neutral slope in *G. soja*

β -conglycinin
-conglycinin

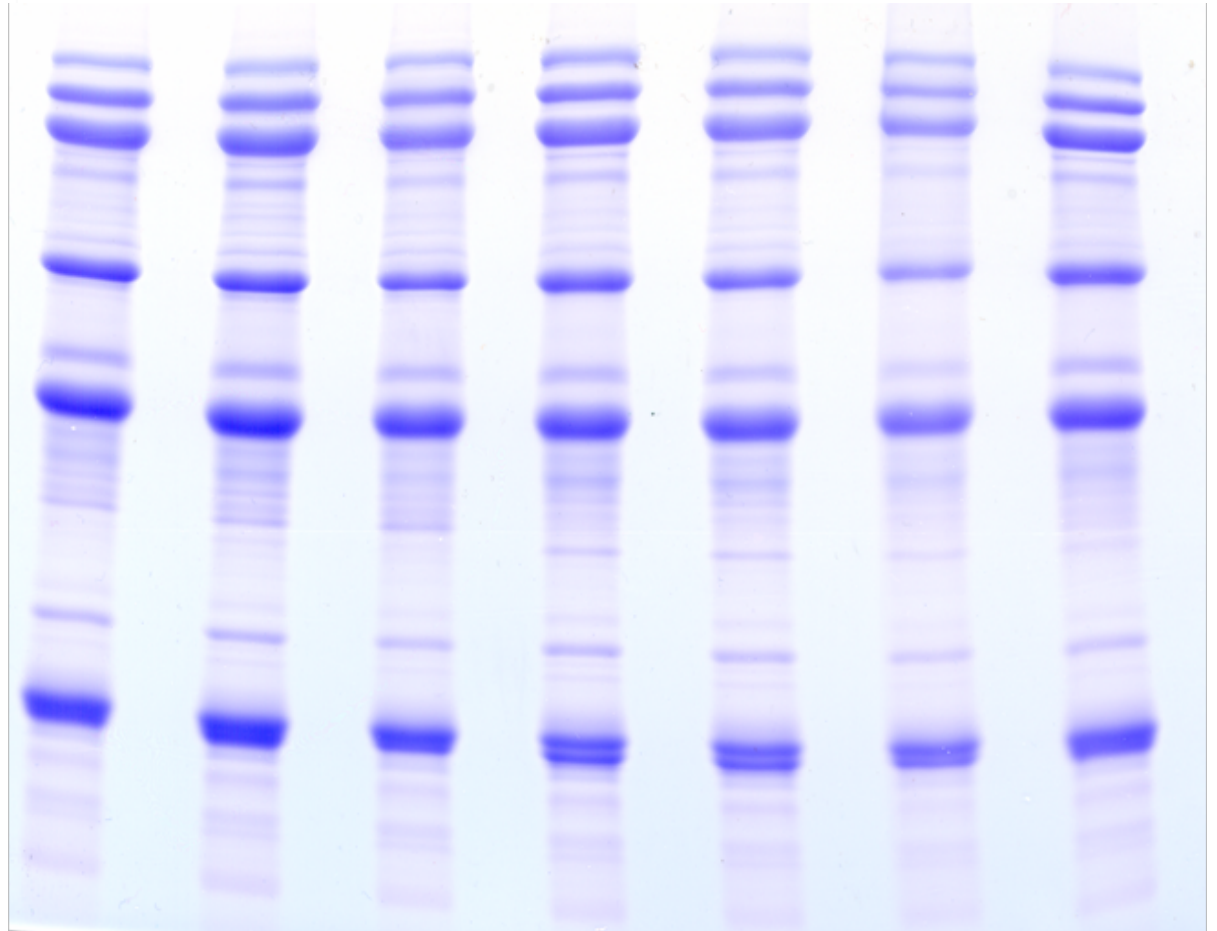
α'
 α
 β

Acidic

C

Glycinin
Glycinin
n

Basic





Relationship between 7S and 11S

- 11S contains about 3-4 times more Met and Cys than 7S
- Soybeans differing in 11S may lead to improvements in S-amino acids.

QTL for 7S and 11S

Trait	Marker	MLG	Position (cM)	LOD Score	R2(%)
7S	Satt461	D2	99.4	2.1	11.6
	Satt249	J	64.5	2.3	10.3
11S	Satt461	D2	99.4	2.3	19.5
	Satt564	G	15.1	3.7	21.8
	Satt191	G	23.2	3.3	18.6
	Satt292	I	2.0	2.0	9.5

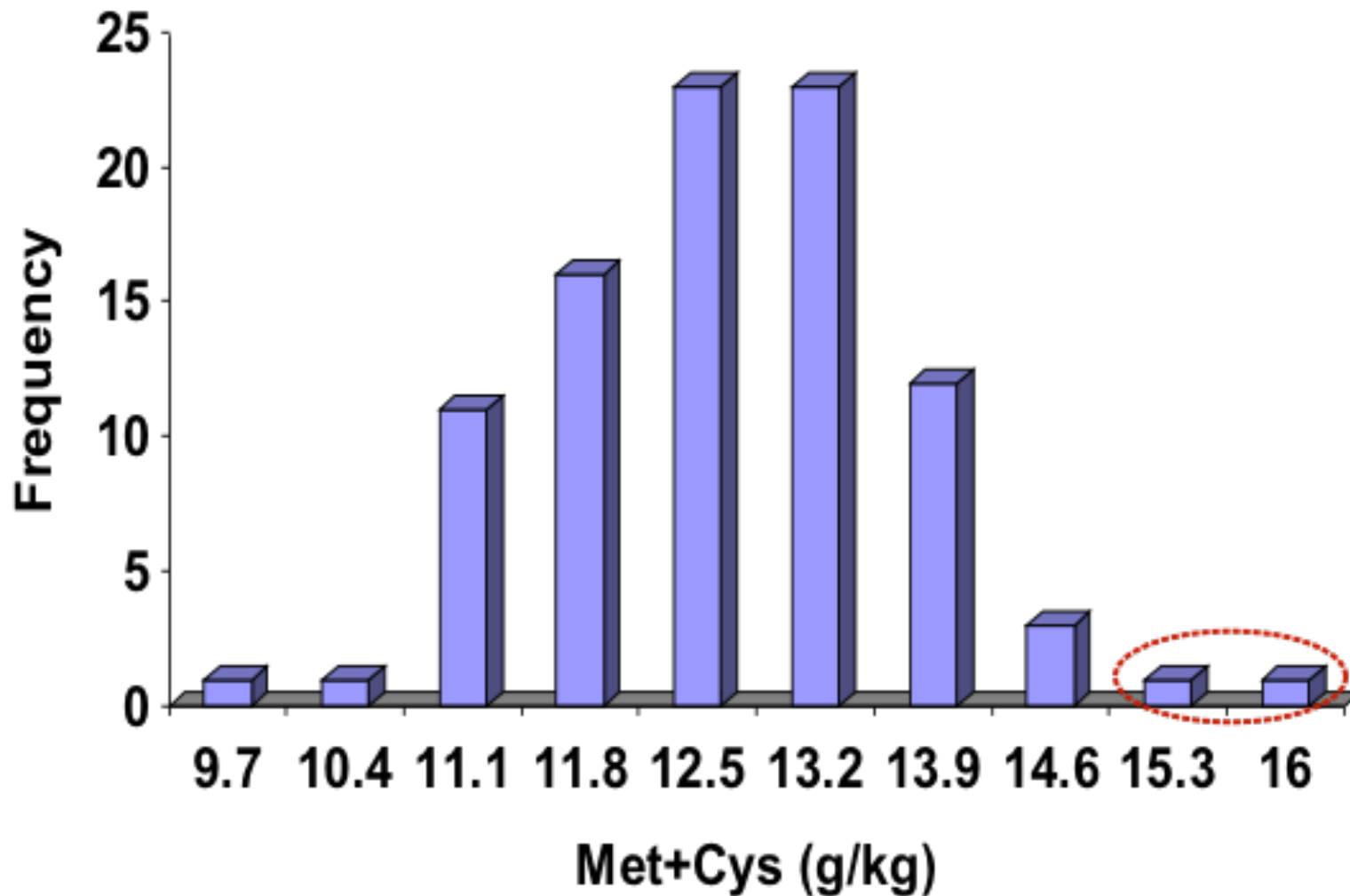
QTL associated with Met

Marker	MLG	Position (cM)	LOD Score	R2(%)
Satt252	F	16.1	2.8	15.2
Satt564	G	14.3	3.4	24.5
Satt590	M	7.8	2.4	22.9

QTL associated with Cys

Marker	MLG	Position (cM)	LOD Score	R2(%)
Satt436	D1a	70.7	2.6	9.5
Satt252	F	16.1	2.6	12.4
Satt235	G	21.9	2.8	12.6
Satt427	G	51.7	3.5	13.8

Frequency distribution for Met+Cys





Protein Quality Germplasm Line Developed & Released

(Panthee and Pantalone, 2006, Crop Sci. 46: 2328-2329)

- **TN04-5321**: Higher protein, and higher S-containing amino acids
- Other breeders use as parent

Soybean Seed Amino Acid Content QTL Detected Using the Universal Soy Linkage Panel 1.0 with 1,536 SNPs

Benjamin D. Fallen^{1,2*}, Catherine N. Hatcher³, Fred L. Allen², Dean A. Kopsell², Arnold M. Saxton², Pengyin Chen⁴, Stella K. Kantartzi⁵, Perry B. Cregan⁶, David L. Hyten^{6,7}, and Vincent R. Pantalone²

- Use the Universal Soybean Linkage Panel
- Expand knowledge of amino acid QTL



Amino Acid QTL F5:7-derived Population of 270 RIL of Essex X Williams 82

• Essex

- genetic background of many S. U.S. varieties

Williams 82

- genetic background of many N. U.S. varieties

- Determine native range of a.a. found in the genetic base of modern U.S. varieties
- Helps identify potential progress breeders can make within elite pool
- Likely need to access other germplasm sources



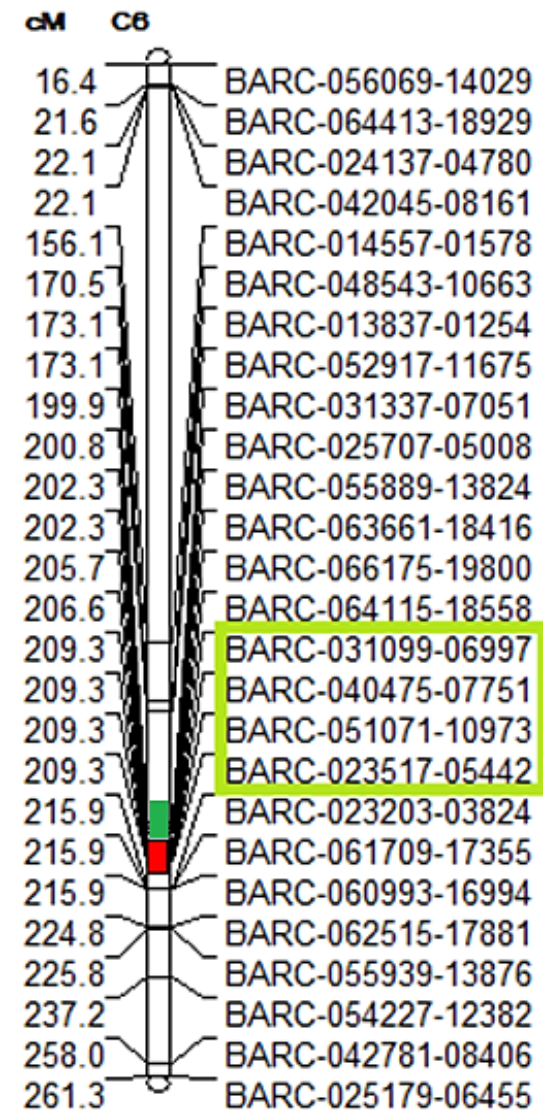
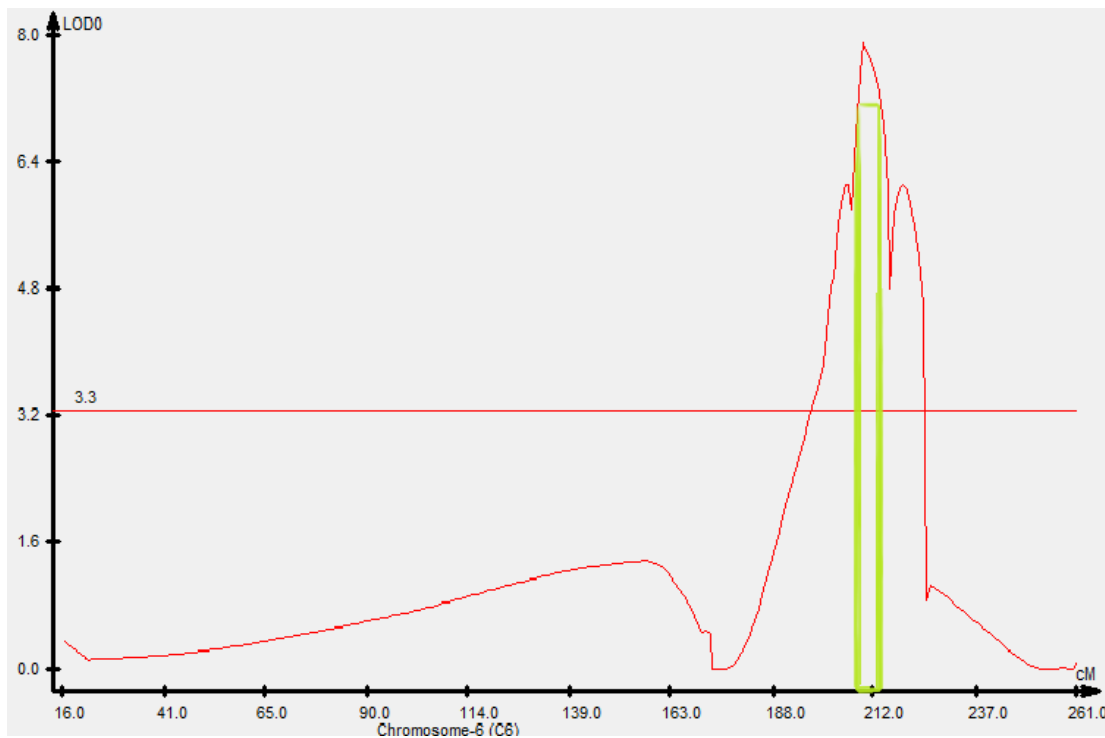


QTL Analysis

- 1,000 permutations were performed on each amino acid for all chromosomes to establish a LOD threshold
- Marker order and position were obtained using R/qtl
- Multiple-QTL Mapping (MQM) and R/qtl were used for QTL mapping

A 1.5-LOD support interval (Broman and Saunak, 2009) was estimated for LGs C2 and L to determine a.a. QTL that were not maturity or growth habit for LGs C2 and L .

LOD confidence interval illustration



(Smallwood et al., 2014)

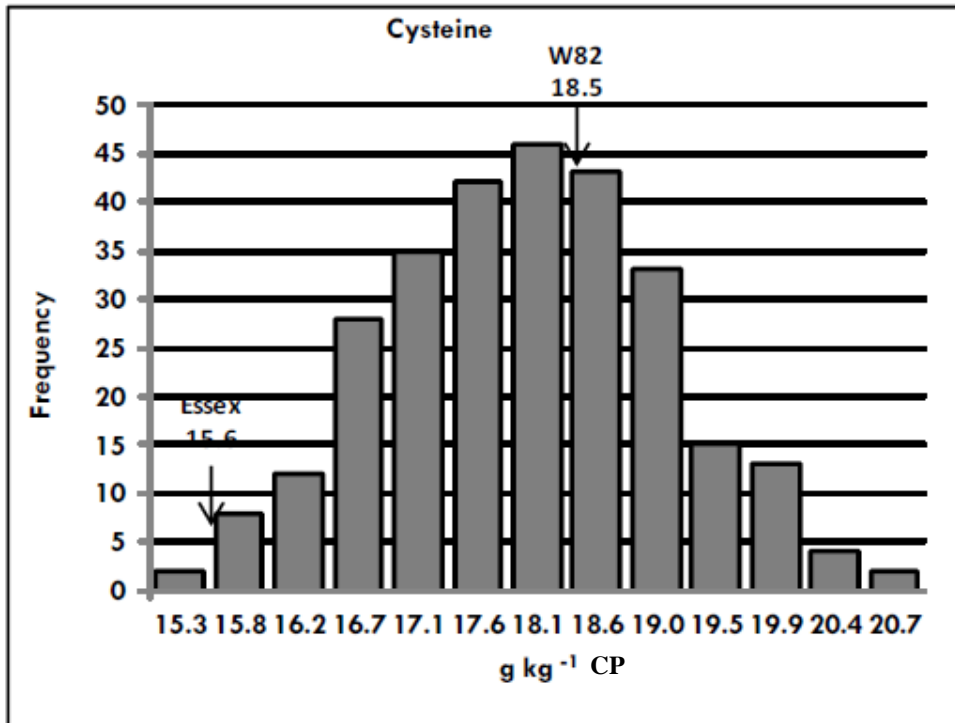


Figure 1. Histogram of cysteine concentrations (g kg^{-1} crude protein) from 282 $F_{5:9}$ -derived RILs of Essex x Williams 82 grown in Knoxville, TN, Stuttgart, AR, and Harrisburg, IL in 2009.

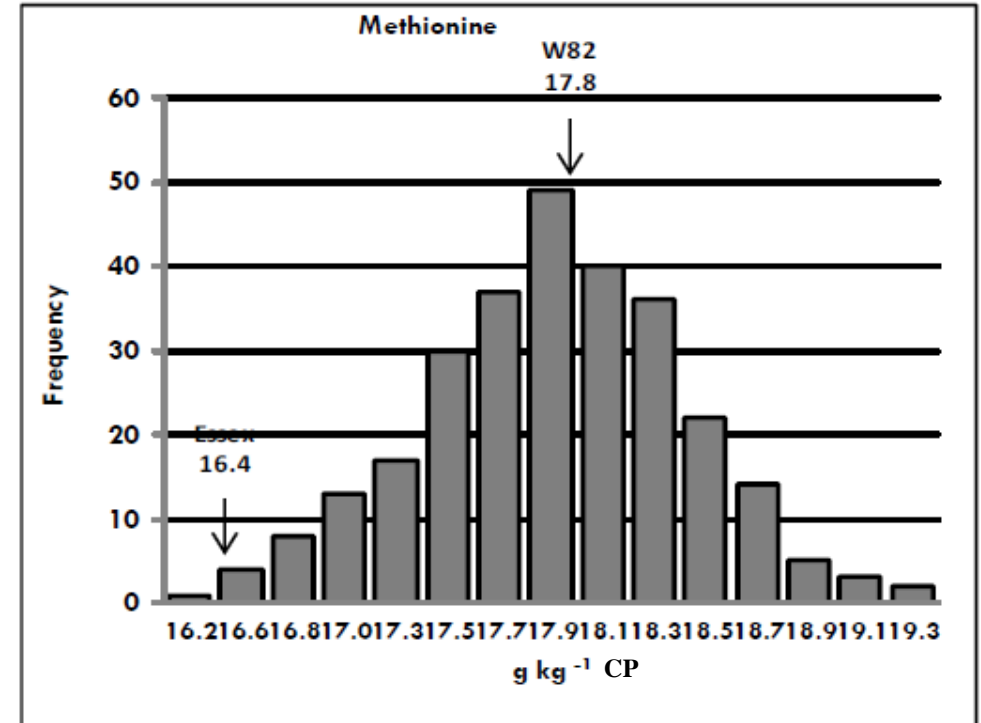


Figure 2. Histogram of methionine concentrations (g kg^{-1} crude protein) from 282 $F_{5:9}$ -derived RILs of Essex x Williams 82 grown in Knoxville, TN, Stuttgart, AR, and Harrisburg, IL in 2009.

Selected nutritionally important amino acid QTL in an F5:9-derived RIL population of Essex 86-15-1 x Williams 82-11-43-1 grown in TN, AR, and IL.

Chr. 1-10

SNP Marker	Amino acid	C hr	M LG	Location (cM)	LOD	R2 (%)
ss107923612	Val	5	A1	145.5	3.4	6
ss107912624	Trp	9	K	0.0	3.1	5.7
ss107920438	Tyr	10	O	110.2	3.1	5.7


Chr. 11-20

SNP Marker	Amino acid	Ch r	ML G	Location (cM)	LOD	R2 (%)
ss107912768	Thr, Val	13	F	0.0	3.6, 3.8	6.4, 6.7
ss107917837	Arg, Met, Thr, Val	13	F	4.9	3.0 to 4.2	5.2 to 7.4
ss107912633	Arg, Val	13	F	21.5	3.5, 3.0	6.1, 5
ss107920654	Arg, Val	13	F	40.7	4.5	7.9, 7.9
ss107924703	Trp	13	F	52.1	3.8	6.5
ss107929220	Cys	20	I	133.4	2.9	6




Genetic Control of Soybean Amino Acid in Two Genetically Connected Populations – Dr. Carrin Carlson (NCSU, Dr. Cardinal)

- NC-Roy x Prolina (277 F4 RIL) and NC-Roy x NC-106 (270 F4 RIL)
- estimate heritability of and genetic correlations
- detect amino acid QTL

- 
- Genomic regions on four linkage groups were significant for a majority of the a.a.
 - Chr 3(N), Chr 6(C2), Chr 9(K), and Chr18(G).
 - A Cys content QTL on Chr10(O) (88.1 – 93.2 cM) provides an opportunity to increase Cys content alone

(Carlson, 2011)


- 
- Significant, positive genetic correlations greater than 70% between concentrations of protein and all amino acids, except Cys, His, and Trp
 - Implying opportunity to indirectly select for a.a. by selection for total protein.
 - lack of ability to increase some a.a. while maintaining consistent levels of protein and remaining amino acids.

(Carlson, 2011)




Dr. Warrington, UGA (Boerma)

- 140 F5-derived RILs from a ‘Benning’ × ‘Danbaekkong’
- Each amino acid sample corrected as a percent crude protein content
- QTL were detected for crude protein (cp), Lys/cp, Thr/cp, Met/cp, Cys/cp, and Met+Cys/cp using DNA markers.

- 
- Large-effect QTL on chr 20 inherited from Danbaekkong explained 55% of the phenotypic variance for crude protein
 - The Danbaekkong protein QTL was found to be associated with reduced levels of each of the amino acids

(Warrington, 2011)

- 
- concentrations of S-containing amino acids may be improved by introgressing Danbaekkong a.a. allele on chr 10 and Benning alleles at QTL on chr 6, 14, and 18
 - -- while still increasing the level of protein with the Danbaekkong QTL at chr 20

(Warrington, 2011)

Mean amino acid composition (g kg⁻¹ of crude protein) from 302 F5:11-derived RIL of Essex x Williams 82 grown in Knoxville, TN; Springfield, TN; and Milan, TN.

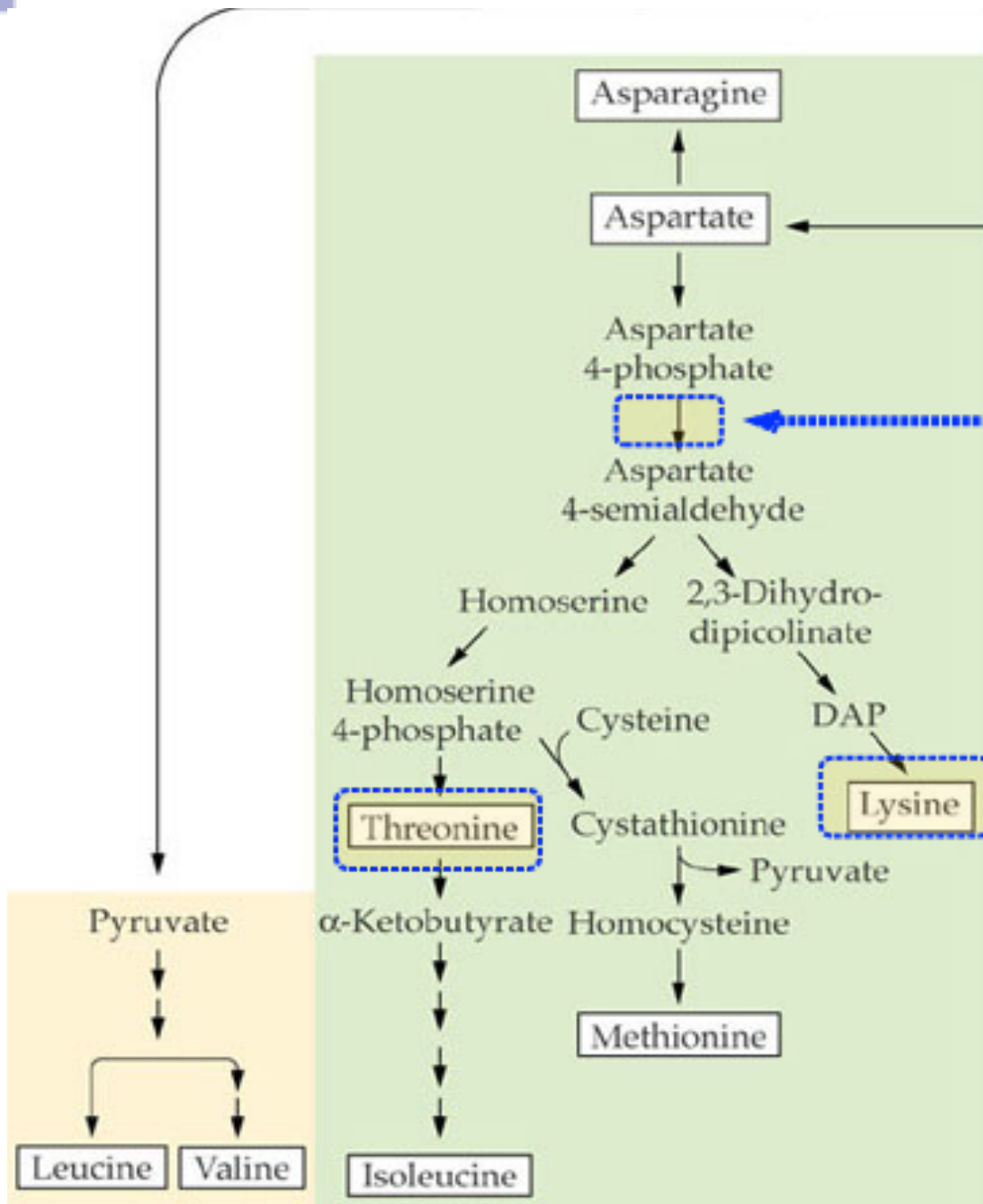
Trait	Min	Mean	Max	LSD0.05	h2 (%)
Cysteine	11.5	18.8	21.3	1.7	29.8
Methionine	12.9	17.0	18.1	1.0	53.9
Lysine	63.2	70.9	73.7	1.8	59.3
Threonine	37.5	42.1	43.9	1.1	78.0
Tryptophan	10.2	11.7	12.7	0.5	81.6

Combined location analysis of **QTL** using composite interval mapping for seed **amino acid composition** in 302 F5:11-derived RIL of Essex x Williams 82 grown in Knoxville, TN; Springfield, TN; and Milan, TN.

Trait	Chr	<u>Molecular Marker</u>	Loc (cM)	LO D	Confidence Interval of QTL position	R2 (%)	Effect g kg ⁻¹
Cysteine	Gm 9	Gm09_1723633_G_A	0.0	5.8	0.0-4.0	6.8	0.08(W)
Cysteine	Gm 13	Gm13_38249824_T_C	199.0	4.4	196.0-201.9	4.5	0.06(W)
Lysine	Gm 6	Gm06_45433980_G_A	57.2	6.4	52.0-60.3	7.4	0.18 (E)
Lysine	Gm 7	Gm07_14773717_G_T	48.7	3.4	38.2-87.8	5.6	0.14(W)
Lysine	Gm 9	Gm09_40970267_C_T	60.0	5.9	56.6-64.9	8.6	0.17(E)
Lysine	Gm 13	Gm13_35823484-A_G	184.0	8.8	181.2-187.0	12.0	0.20(W)
Lysine	Gm19	Gm19_42089062_C_T	196.0	4.3	178.0-213.0	5.4	0.14 (W)

Combined location analysis of **amino acid QTL** (continued)

Trait	Chr†	<u>Molecular Marker</u>	Loc § (cM)	LO D	Confidence Interval of QTL position	R2 (%)	Effect # g kg-1
Methionine	Gm 9	Gm09_1723633_G_A	0.0	6.0	0.0-4.85	7.4	0.06 (W)
Methionine	Gm 13	Gm13_35823484_A_G	184.0	5.4	183.0-191.9	6.9	0.06(W)
Methionine	Gm 18	Gm18_2020495_C_T	5.1	3.1	5.0-12.0	4.0	0.05 (W)
Threonine	Gm 6	Gm06_45871481_C_T	58.0	4.8	51.0-83.0	5.7	0.10 (E)
Threonine	Gm 7	Gm07_9913651_T_C	43.3	4.9	41.7-69.6	6.9	0.09 (W)
Threonine	Gm 9	Gm09_38958410_A_G	64.1	5.4	59.0-66.0	8.3	0.10(E)
Threonine	Gm 13	Gm13_35370448_C_T	183.0	10.5	181.2-187.0	14.3	0.14 (W)
Tryptophan	Gm 6	Gm06_44116624_T_C	54.1	5.6	51.0-60.0	7.3	0.05(E)
Tryptophan	Gm 13	Gm13_36316916_C_T	188.3	5.3	182.0-204.6	5.0	0.03 (W)



Are QTL detecting some of the key enzymes in the common enzymatic pathway towards production of Lysine and Threonine?

... further research for discussion ideas tomorrow.



Remarks

- Perfect SNP markers accelerate breeders' introgression
 - Nothing comparable exists for amino acids, *yet*
- Detection of QTL for 7S and 11S
 - needs confirmation – no cq-QTL exist in Soybase
- Discoveries of Met and Cys QTL
 - Validated one released germplasm – more needed
- Amino acid QTL
 - Need confirmation – no cq-QTL exist in Soybase
 - accelerate protein quality breeding of U.S. soymeal



Thank you

