Breeding high oleic non-GMO soybeans

J. Grover Shannon – MU Soybean Breeding

Kristin D. Bilyeu- USDA Research Molecular Biologist

Anh Pham- PHD student

Jeong Lee- Kyungpook Nat'l University, Daegu, S. Korea

Many collaborators- USB funded oil project

Soybean Composition

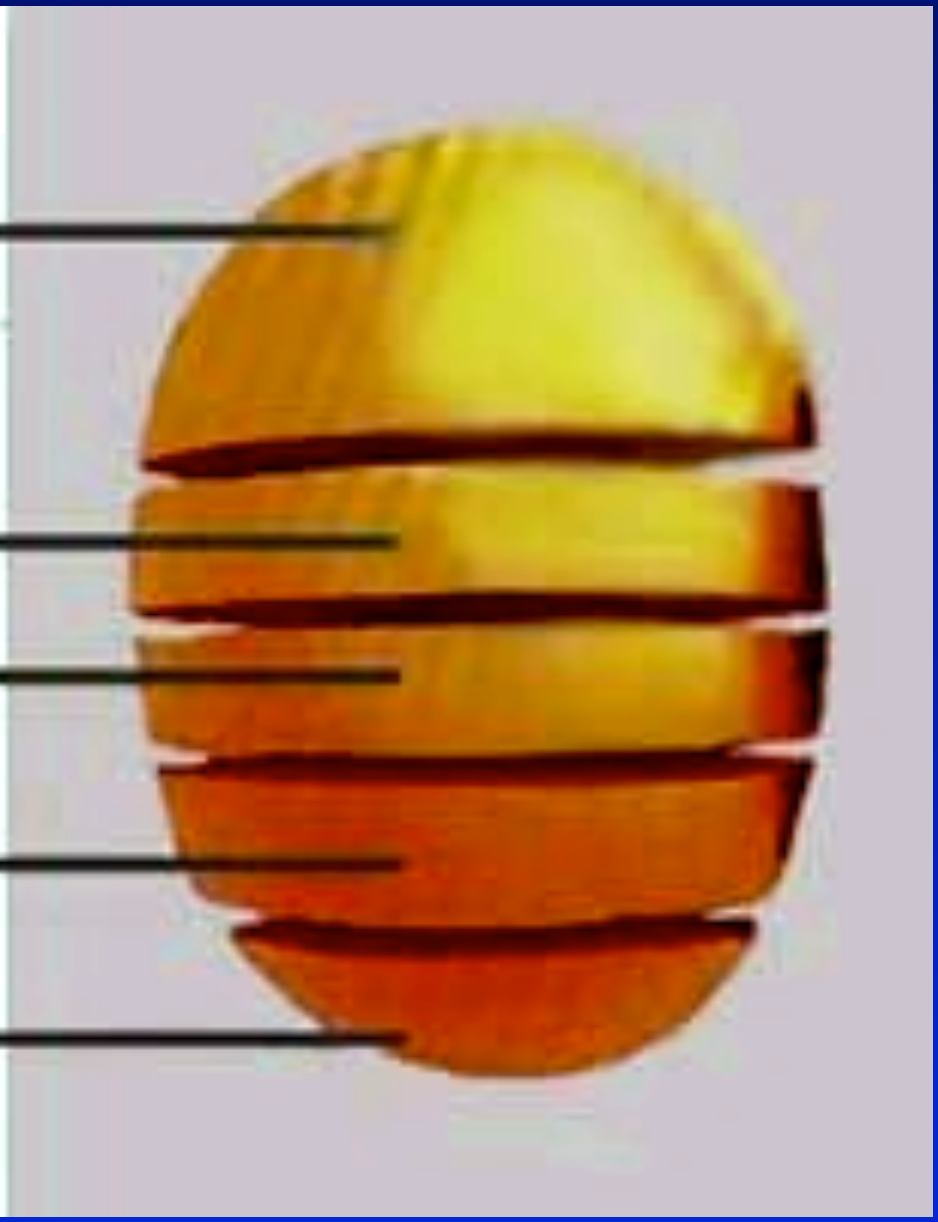
38% Protein

15% Soluble Carbohydrates, (Sucrose, Stachyose, Raffinose, others)

15% Insoluble Carbohydrates (Dietary fiber)

18% Oil (.05% Lecithin)

14% moisture ash/other



Soybeans Are Not Perfect

Limited in food, feed and industrial uses because of:

1. Beany flavor
2. Indigestible carbohydrates
3. Anti-nutritional factors
4. Low oxidative stability of oil
5. Deficiency of AAs cystiene & methionine

UNITED SOYBEAN BOARD
BETTER BEAN INITIATIVE

DEVELOP SOYBEANS WITH BETTER OIL AND MEAL TRAITS TO INCREASE DEMAND FOR U.S. SOYBEANS

OLEIC ACID- One of 5 Fatty Acids in Soy Oil

Palmitic acid (16:0)	- 11%	Saturated
Stearic Acid (18:0)	- 4%	
Oleic Acid (18:1)	- 23%	Unsaturated
Linoleic Acid (18:2)	- 54%	
Linolenic Acid (18:3)	- 8%	
	100%	

Most Desired Soybean Oil Phenotype

Saturates (16:0 +18:0) 15% to < 7%

Oleic acid (18:1) 24% to > 55%

Linolenic acid (18:3) 8% to < 3%

Soybean oil and the *trans* fat rap

Mac and cheese

- To make soy oil more functional it is hydrogenated
- Hydrogenation increases oil stability but **creates trans fat which is heart unhealthy**

Soy-oil with >55% Oleic Acid will

- Increase heat stability, taste & shelf-life
- Have more food applications
- Reduce hydrogenation & trans-fats
- Improve soy-diesel, lubricants
-
- More use in pharmaceuticals & cosmetics


Canola
Soybean
Olive
Pure
Wesson
100% NATURAL
Canola Oil
0g Trans Fat
No Preservatives
Cholesterol Free
24FLOZ(1.5PT)0.7L
PAMPA
Pure
Soybean Oil
& Extra Virgin
Olive Oil
NET WT. 8.5 FL.OZ.
250ml
Best Choice
EXTRA VIRGIN
OLIVE OIL
PRODUCT OF ITALY
COLD PRESSED
NET 17 FL OZ (1 PT 1 OZ) 503mL

Non-GMO sources for developing high oleic acid

- **N98-4445A:** 60% oleic six genes- low yield and unstable across growing environments –
- **M23:** 40-50% oleic – Patented

  - Single recessive gene
  - lower yield, somewhat stable

Six QTLs confirmed for Oleic acid in N98-4445

- **N00-3350 derived from N98 used in mapping genes**

LG	R^2	Marker
A1	4%	Satt211
D2	6%	Satt389
G	13%	Satt394
G	7%	Satt191
L	9%	Satt418
L	25%	Satt561

  - (Monteros & Boerma, Crop Sci (2009)

Once we had the markers-breeder collaborators began using marker assisted backcrossing to introduce the six high oleic genes into adapted lines

Nguyen lab- MO- S. Dak. Minn., & N. MO

Boerma lab- GA- S. MO, AR, TN, NC, GA

Puerto Rico for year round back crossing

Influence of Temperatures at Seed Fill on Desired Fatty Acid Composition

	Best Temperature
Palmitic acid	Little Effect
Stearic acid	Little Effect
High Oleic Acid	Warmer
Low Linolenic acid	Warmer

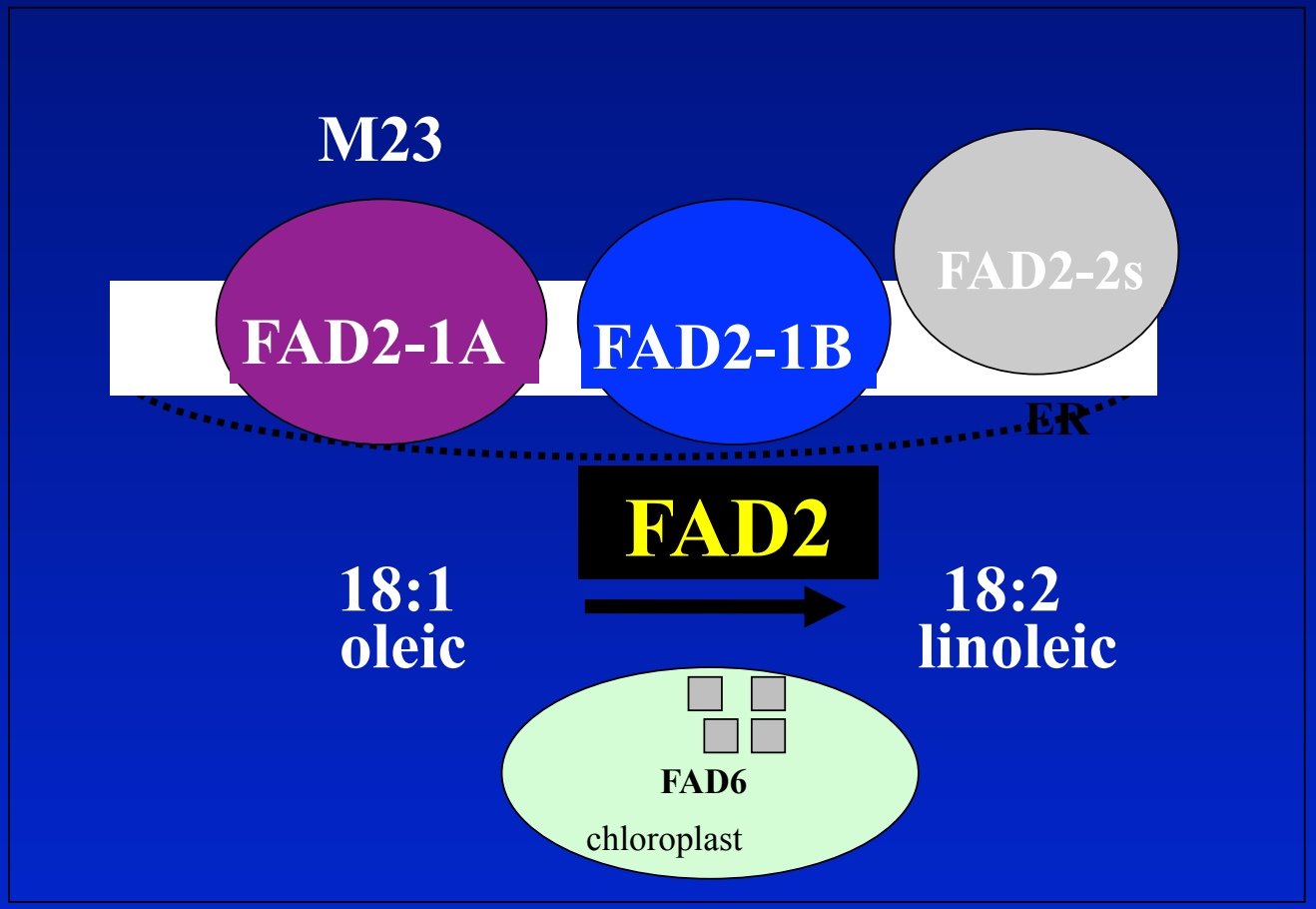
Effect of Environment on Oleic Acid Content of N98-4445

	% Oleic
Mississippi- very warm	70
Southern Missouri- warm	60
Central Missouri- cooler	45
Central Iowa- much cooler	35

Unless stable oleic acid genes can be found without the influence of temperature, mid oleic acid strains will need to be:

- **Produced in warmer regions**
- **Early in maturity**
- **Planted at earlier dates to have pod fill stage when temperatures are warmest**

The suspects: FAD2-1A and FAD2-1B



Plant introductions with elevated Oleic acid

Most soybeans have about 23% oleic acid

About 50 plant introductions have higher oleic acid content, 30-45%

Useful and fewer genes for improving oleic content and less variation in 18:1 over locations

Higher Oleic Plant Introductions

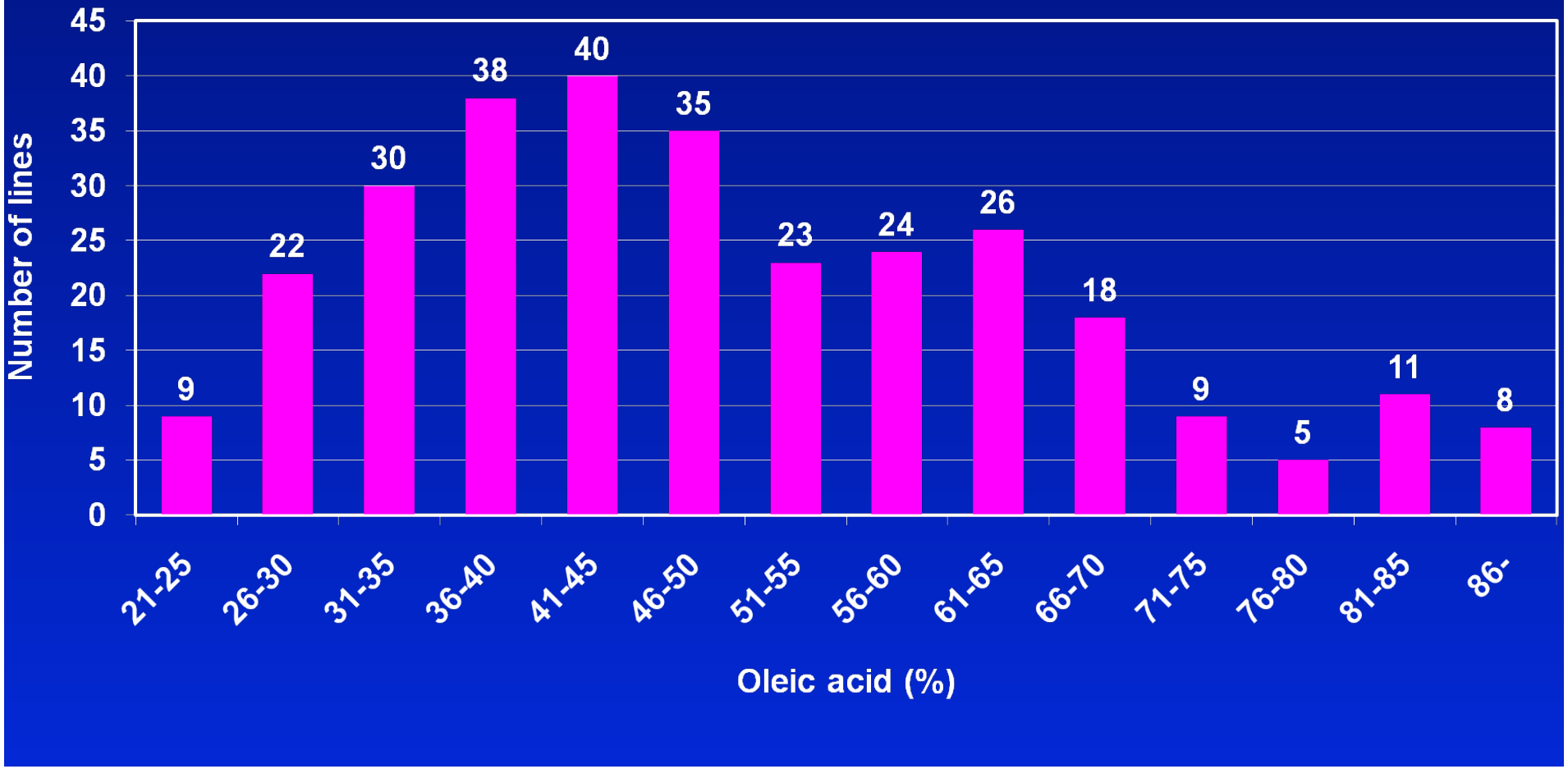
- **Could have a simple gene for high oleic acid**
- **Could be more stable across growing conditions**
- **Genes from PIs with 35 to 40% oleic acid could be combined to reach > 55% oleic acid ?**

Combining genes affecting % 18:1 from M23 (one gene), and N98-4445 (one or more of the six genes?) have generated phenotypes with >70% oleic acid in MO, but only about 55% in Iowa.

Iowa State U.- Fehr lab

M23 x PI283327

(n=299, Oleic acid range: 21.9-86.6%, Pop. mean: 49.2%)



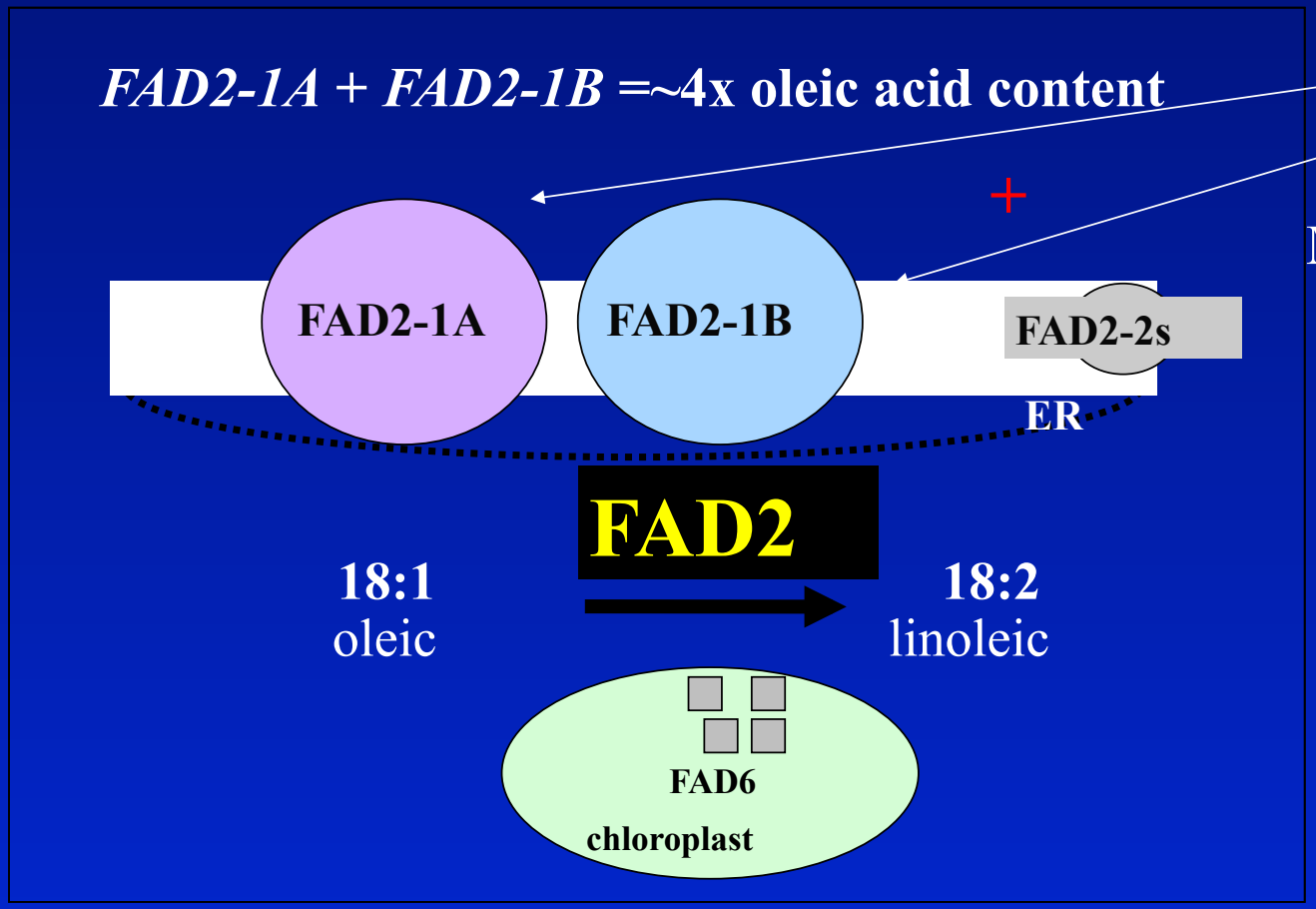
Reverse genetics targeting *GmFAD2-1A* (TILLING)

M23- 45% 18:1- 100 kb deletion- radiation
17D- 30% 18:1- one base pair deletion- EMS

Sources of mutant *FAD2-1* alleles

- *FAD2-1A*: Chromosome 10
  - M23 (~100 kb deletion on chromosome 10); Bay background
  - 17D (S117N); Williams 82 background
- *FAD2-1B*: Chromosome 20
  - PI 567189A and PI 578451 (I143T); group IV
  - PI 283327 and PI 210179 (P137R); group V

Combinations of mutations in *FAD2-1* genes create Non-GMO high oleic acid soybeans



FAD2-1aabb **accumulates 80% of 18:1**

Pham et al., 2010. Plant Biol. 195:

17 D (FAD2-1A) x PI 283327 (FAD2-1B) F2 Seed chips

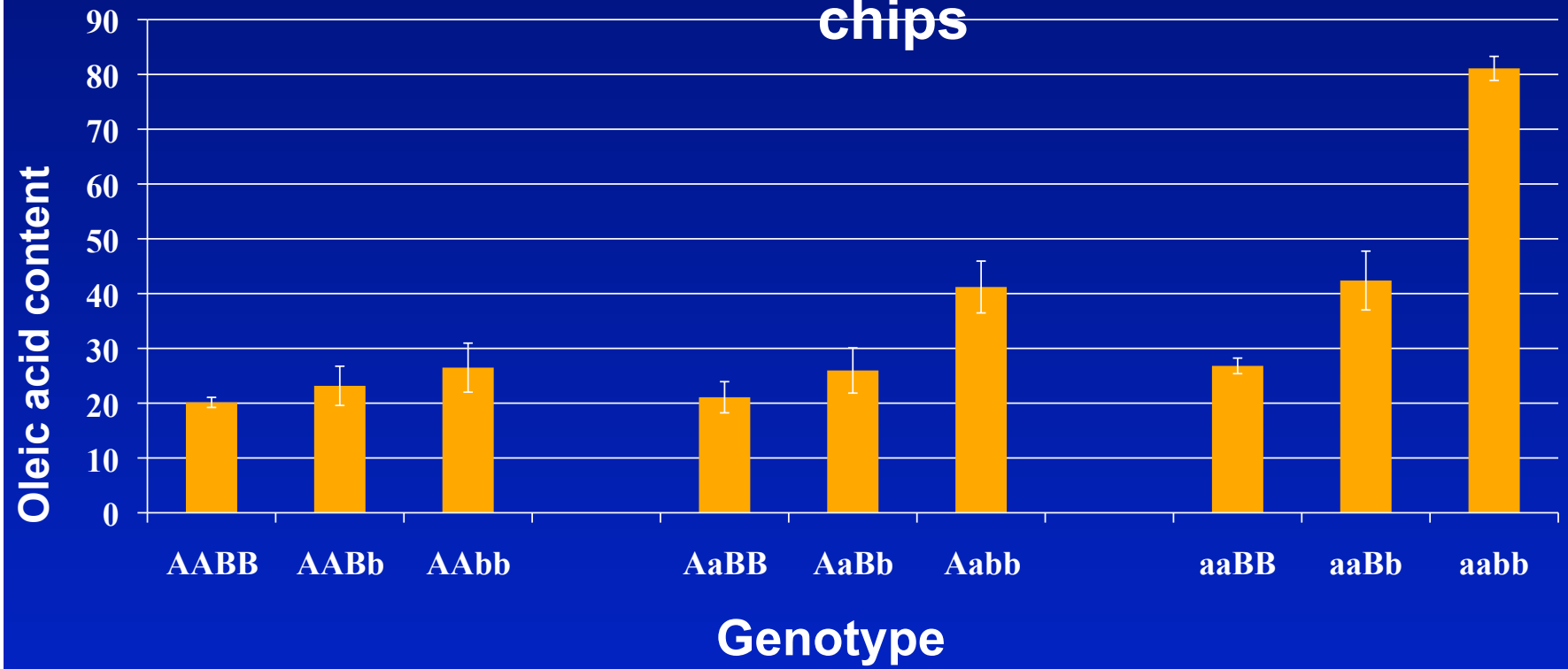
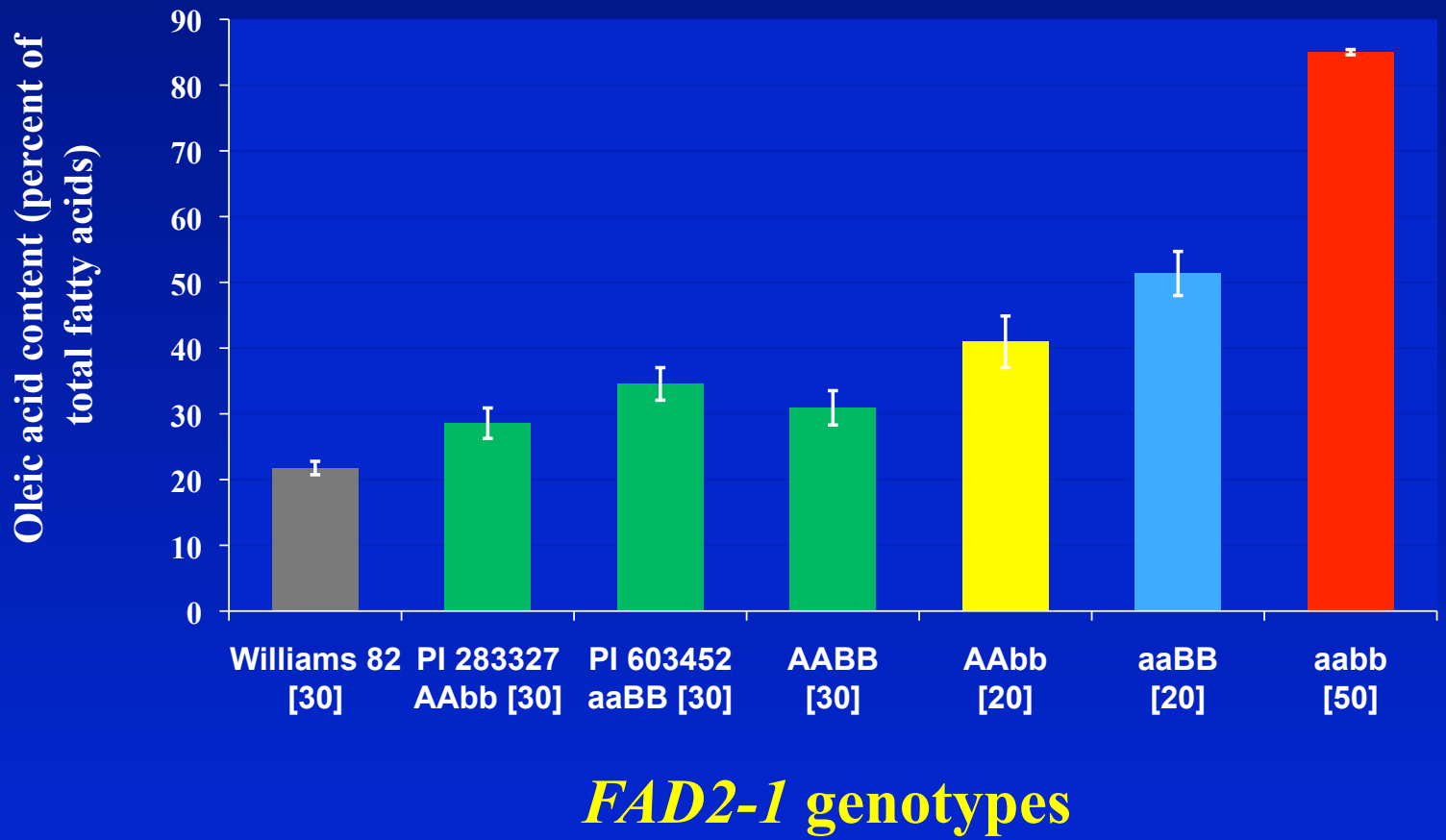


Figure 1. Seed oleic acid phenotype and *FAD2-1* genotype association analyses of soybean lines of the cross PI603452 (FAD2-1A) x PI 283327 (FAD2-1B) grown in Columbia and Portageville MO in summer 2010



Oleic stability over 8 environments* for two 80% lines from M23 x PI 283327, 2010

	Missouri				
	Col	Pville	Knox, TN	Stone, MS	Hi-Lo
S08-14707 aabb	75	80	78	81	6
S08-14717 aabb	76	82	82	80	6
PI 283327- parent	23	28	25	30	7
M23- parent	44	42	52	59	17
N98-4445A- check	47	56	64	63	17

Bilyeu, Shannon, Pantalone, Gillen two planting dates per location

What about stability of 18:1 further north

An FAD2-1A x FAD2-1B 80% oleic line from 17D x PI283327 grown in South Dakota in 2010 was 69% oleic, but probably late group III-group IV maturity but matured without frost damage.

Question- How much higher would oleic acid be if seed was from a line in a maturity adapted to S.D.?

Typical FA profile of high 18:1 F_2 seed used in 2010 MO, GA, NC, TN & AR crosses

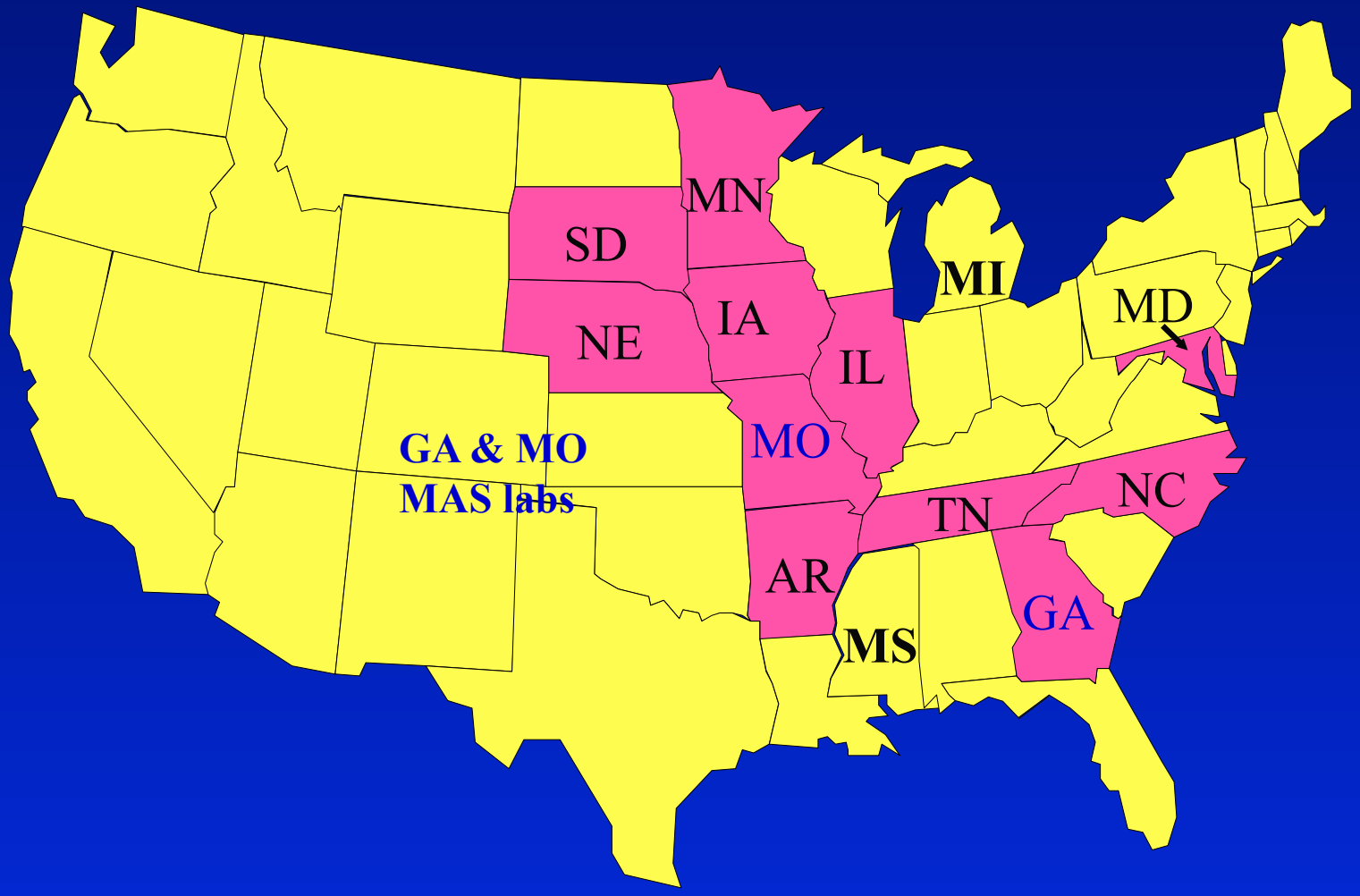
Pedigree (87.5% adapted background)	16:0	18:0	18:1	18:2	18:3
S05-11482 x F2 (17D x S08-14788)	8.4	3.3	82.3	2.4	3.7
S06-10572RR x F2 (17D x S08-14788)	7.8	3.2	83.7	2.0	3.4
S06-4649RR x F2 (17D x S08-14788)	7.5	2.8	84.2	2.0	3.4
Average soybeans	11	4	24	53	8

Do these FAD2 mutations affect yield?

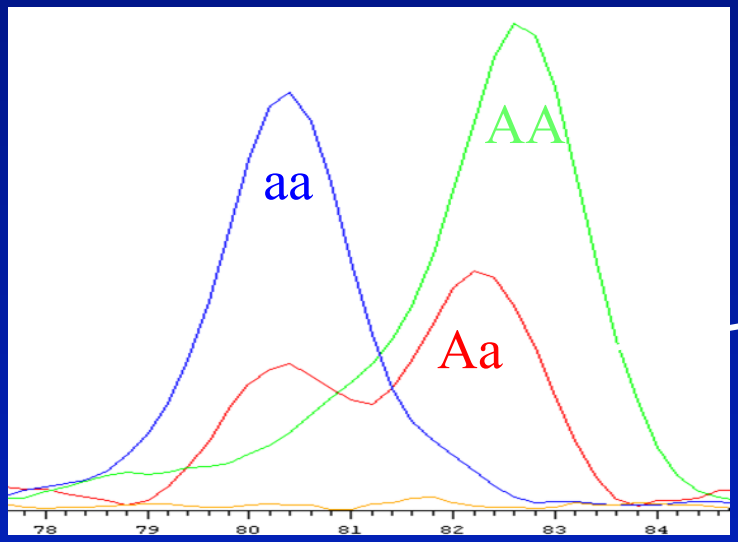
- Hoshino et al., 2010 (Breeding Science 60:419-425)
  - M23- FAD2-1A 100 kb deletion- large deletion affects yield, thus any cross combination with M23 will likely affect yield.
  - KK21- (FAD2-1A) x B12 (FAD2-1B) single base pair deletions – 80% oleic with no affect on yield
  - 17D and PI603452- (FAD2-1A) and PI283327 (FAD2-1B_- single base pair deletion should not affect yield?

States Working on Oil Traits

Demand 8 to 20Mil Acres of Hi 18:1



Molecular marker assays for accelerated plant breeding



- Genotype selections can be done early
- Less effort and investment for better results

Sources of HI oleic- GMO

- Pioneer-DuPont- “Plenish” on market in 2012
- Monsanto- “Vistive gold”
- Good yield and stable over environments

THANK YOU

- **United Soybean Board for funding for the Better Bean Initiative Projects!**

- **QUESTIONS?**