Breeding high oleic non-GMO soybeans

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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%
- Stearic Acid (18:0) - 4%
- Oleic Acid (18:1) - 23%
- 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

- 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
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

<table>
<thead>
<tr>
<th>LG</th>
<th>$R^2$</th>
<th>Marker</th>
</tr>
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<tbody>
<tr>
<td>A1</td>
<td>4%</td>
<td>Satt211</td>
</tr>
<tr>
<td>D2</td>
<td>6%</td>
<td>Satt389</td>
</tr>
<tr>
<td>G</td>
<td>13%</td>
<td>Satt394</td>
</tr>
<tr>
<td>G</td>
<td>7%</td>
<td>Satt191</td>
</tr>
<tr>
<td>L</td>
<td>9%</td>
<td>Satt418</td>
</tr>
<tr>
<td>L</td>
<td>25%</td>
<td>Satt561</td>
</tr>
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</table>

(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

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Best Temperature</th>
<th>Little Effect</th>
<th>Warmer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stearic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Oleic Acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Linolenic acid</td>
<td></td>
<td></td>
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</tbody>
</table>
Effect of Environment on Oleic Acid Content of N98-4445

<table>
<thead>
<tr>
<th>Location</th>
<th>% Oleic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mississippi- very warm</td>
<td>70</td>
</tr>
<tr>
<td>Southern Missouri- warm</td>
<td>60</td>
</tr>
<tr>
<td>Central Missouri- cooler</td>
<td>45</td>
</tr>
<tr>
<td>Central Iowa- much cooler</td>
<td>35</td>
</tr>
</tbody>
</table>
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

- FAD2-1A
- FAD2-1B
- FAD2-2s
- M23

ER

18:1 oleic

FAD2

18:2 linoleic

FAD6
chloroplast
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)

Oleic acid $18:1$ \[ \xrightarrow{\text{FAD2}} \] linoleic acid $18:2$  

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

GmFAD2-1A \[ \xrightarrow{\text{FAD2}} \] GmFAD2-1B
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

\[ FAD2-1A + FAD2-1B = \sim 4x \text{ oleic acid content} \]

MUTATIONS

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

Oleic acid content

Genotype

AABB  AABb  AAbb  AaBB  AaBb  Aabb  aaBB  aaBb  aabb
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

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

*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 F₂ seed used in 2010 MO, GA, NC, TN & AR crosses

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

GA & MO
MAS labs
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?