The Complex Tale of the High Oleic Acid Trait in Peanuts

Noelle Barkley, USDA ARS PGRCU
Claire Klevorn, NC State University
Keith Hendrix, USDA ARS Market Quality & Handling Research
Lisa Dean, USDA ARS Market Quality and Handling Research

Peanut Germplasm Collection
~10,000
- Wild and Cultivated peanut germplasm
- 9,224 cultivated; 608 wilds

Responsibilities
- Regeneration
- Distributions
- Breeding, genomics, evaluation
- Characterization
  - genotyping and phenotyping
  - GBS, markers, association
  - Breeding

Regeneration targets
- Storage
  - 10-15 years to avoid loss
    - 660-1000 accessions regenerated / year
      - Base funding USDA
      - Help from peanut breeders
      - Grant to support larger scale germplasm regeneration and genotyping and phenotyping
  - Walters et al. 2005 Seed Science Research
    - P50
      - 780 accessions = 25 years in -18C
      - 372 cultivated accessions / year
      - Wilds = no data

Genetic Diversity

Botany of the Peanut
- Arachis hypogaea (cultivated)
  - “hypogaea” greek for underground
  - Legume Family
  - Originated in South America
  - Self pollinates
  - 1-2 % flowers make pod/seeds
  - indeterminate
  - 90-150 days maturity

- Market types
  - Runner- PB
  - Virginia – large seeded
  - Spanish – higher oil – sweets
  - Valencia – 3-5 seeds/pod

Heterogeneity/Maturity
Peanut

- *Arachis hypogaea*
- Allotetraploid \(2n = 4x = 40\)
- Source of protein, folate, tocopherol, polyphenolics, fiber, and oil
- Oil content ranges from \(~40\) to \(~55\%\)
  - 90% of oil derived from palmitic \((16:0)\), oleic \((18:1)\), and linoleic \((18:2)\) fatty acids
  - 8-12 fatty acids total
- Quality depends on oil content
  - All oils prone to oxidation
    - Polyunsaturated fats more prone to oxidative rancidity

High oleic Peanuts

- Many countries switched to only producing high oleics
- Important seed quality trait
  - Longer shelf life
  - Improved flavor
- Nutrition
  - Mediterranean effect associated with dietary habits improving cardiovascular risk factors (lipoprotein profile, blood pressure, endothelial function, insulin resistance, inflammation, oxidative stress)
  - Antioxidant stability
- Majority high oleic peanuts derived from F435
  - F435 = 80% oleic and 2% linoleic \((O/L = 40)\)
  - O/L ratio > 10 to be considered a high oleic

Comparison of Oils

<table>
<thead>
<tr>
<th>Oil Type</th>
<th>Monounsaturated</th>
<th>Polyunsaturated</th>
<th>Saturated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hi Oleic Peanut</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Olive Oil</td>
<td>90%</td>
<td>10%</td>
<td>0%</td>
</tr>
<tr>
<td>Canola Oil</td>
<td>80%</td>
<td>20%</td>
<td>0%</td>
</tr>
<tr>
<td>Regular Peanut</td>
<td>70%</td>
<td>30%</td>
<td>0%</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>60%</td>
<td>40%</td>
<td>0%</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>50%</td>
<td>50%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Segregation of Oleic Trait

- **Genotype I**
  - \(o_{1}o_{1}o_{2}o_{2}\) Normal
- **Genotype II**
  - \(o_{1}o_{1}o_{2}o_{2}\) High

Fatty acid desaturase gene \((ahFAD2)\)

- 2 homoeologous genes: \(ahFAD2A\) and \(ahFAD2B\)
  - 99% sequence identity \(ahFAD2A\) and \(ahFAD2B\)
    - Enzyme responsible for conversion of oleate to linoleate
- Mutation events occurred at both loci
  - Substitution at \(ahFAD2A\) gene = G448A
  - Insertion in \(ahFAD2B\) gene = 442insA
- Both mutations result in non-functional \(\Delta^{12}\)-desaturase enzyme resulting in higher \(O/L\) ratio in peanut oil
  - Desired high oleic genotype \(ol_{1}ol_{1}ol_{2}ol_{2}\)
Objectives

• Develop high oleic lines with enhanced disease resistance

• Marker Assisted Selection (MAS)
  • Genotype at each developmental stage – parents, F₁, F₂,..., Fₙ
  • High oleic and nematode resistance

• Establish a link between fatty acid content and genotype

Assay Design ahFAD2

Segregation Analysis

<table>
<thead>
<tr>
<th>Cross</th>
<th>O/L Ratio F₂</th>
<th>χ² (15:1)</th>
<th>χ² (3:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fla-07 × A. hypogaea var. hirsuta (o₁o₁o₂o₂ × O₁O₁O₂O₂)</td>
<td>0.85-43.58</td>
<td>2.54</td>
<td>32.86***</td>
</tr>
<tr>
<td>York × A. hypogaea var. peruviana (o₁o₁o₂o₂ × O₁O₁O₂O₂)</td>
<td>0.93-32.42</td>
<td>1.86</td>
<td>15.00***</td>
</tr>
<tr>
<td>Chico × York (O₁O₁O₂O₂ × o₁o₁o₂o₂)</td>
<td>0.92-39.47</td>
<td>0.06</td>
<td>20.18***</td>
</tr>
<tr>
<td>Tifguard × York (o₁o₁O₁O₂ × o₁o₁o₂o₂)</td>
<td>1.14-28.32</td>
<td>1.50</td>
<td>145.20***</td>
</tr>
</tbody>
</table>

*** = difference is statistically significant 0.001 probability

Gas Chromatography

Fatty Acid Distributions

Linoleic 18:2

Oleic 18:1

Palmitic 16:0
ANNOVA - Fatty Acid and Genotype

- Significant Results
  - Selection of parents influenced FA
  - ahFAD2 genotype influenced 16:0, 18:1, 18:2, O/L ratio
  - Pleiotropic effect
  - Genotype did not affect stearic acid 18:0
  - Epistasis revealed ahFAD2A and ahFAD2B
- Additive and dominant affects observed for both loci for the majority of the fatty acids
- Dominance is incomplete
Complications of Oleic Trait

- Purity of the high oleic trait in seed lots
  - Certified seed
  - Breeders stock
  - Germplasm

- Problem for processors and buyers in peanut industry
  - Rely on the oil chemistry for their products
  - RUTF’s – International work

Anecdotal Evidence

- Planting, harvesting, handling by farmers, shellers, industry etc.
  - Impure seed lots frequent detection GC and genotyping
  - USA normal and high oleics planted and produced

- Genotyping F7 populations
  - 5 seeds bulked and extracted of each F7 line
  - Genotyped individuals from each line
    - 1 of 5 different FAD2 genotypes within the bulk
    - Some bulks had 5 different genotypes

- Field study and tracking oil content changes
  - Breeder seed planted
  - Pulled plants every 15 days for pod blasting/maturity and oil characterizing
  - 20-55% of plants not high oleic

Further Anecdotal Evidence

- Outcrossing?
  - 10-30% suggested but highly depending on cultivar and environment

- Additional genes involved?
  - 6 to 13 genes been suggested

- Linked to Seed Maturity

Role of Maturity

Single Seed O/L Ratio by Maturity Class

Streams of Bailey – normal oleic

Streams of Spain – high oleic
High Oleic Trait

- Not as simple as we believed
- Very difficult to maintain pure high oleic seed lots
  - Processing?
  - Maturity?
  - Genetics?
  - Reversion?
  - Outcrossing?
- Difficult to maintain trait when high and normal oleics all in production and processed together
  - Continue to be a problem going forward

Genetic Linkage Map & QTLs

\textbf{ahFAD2 Gene Structure}

- 3,529 bp
  - Intron found in cotton, Arabidopsis, sesame
- CDS: 1140 bp; no introns, protein is 379 A.A.
**Real-Time PCR Assay**

- Developed a PCR assay to distinguish normal and high oleic acid peanuts based on genotype (<1.5 hr)
- Assay works on seed or leaf material
- Detect heterozygotes both within and between genomes

- Target: 442insA - single bp insertion in ahFAD2B and G448A in ahFAD2A
- Utilized A/B genome SNPs that flank mutations in primer design

- Optimized method on samples with known O/L ratios
- High Oleic – Florida-07,York, F435, Georgia-02C
- Normal Oleic – Chico, Virugard, Tifguard, Botanical Varieties

---

**Crosses**

<table>
<thead>
<tr>
<th>Female</th>
<th>Genotype</th>
<th>Resistance &amp; O/L</th>
<th>Male</th>
<th>Genotype</th>
<th>Resistance &amp; O/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida-07</td>
<td>Ol$_1$Ol$_1$ol$_1$</td>
<td>Leaf Spots</td>
<td>Ol$_1$Ol$_1$</td>
<td>A. hypogaea var. hirsuta</td>
<td>Ol$_1$Ol$_1$O/L$_2$ TSWV O/L = 0.95</td>
</tr>
<tr>
<td>York</td>
<td>Ol$_1$Ol$_1$ol$_1$</td>
<td>Leaf Spots</td>
<td>Ol$_1$Ol$_1$</td>
<td>A. hypogaea var. peruviana</td>
<td>Ol$_1$Ol$_1$O/L$_2$ TSWV O/L = 1.9</td>
</tr>
<tr>
<td>Chico</td>
<td>Ol$_1$Ol$_1$O/L$_2$</td>
<td>None</td>
<td>Ol$_1$Ol$_1$</td>
<td></td>
<td>Ol$_1$Ol$_1$ol$_1$ Leaf Spots O/L = 1.0</td>
</tr>
<tr>
<td>Tifguard</td>
<td>ol$_1$ol$_1$O/L$_2$</td>
<td>Nematode; TSWV</td>
<td>Ol$_1$Ol$_1$</td>
<td></td>
<td>Ol$_1$Ol$_1$ol$_1$ Leaf Spots O/L = 1.4</td>
</tr>
</tbody>
</table>

---

**Results**

- MAS to track functional mutations: oleic and nematode resistance
  - Select desirable genotypes in each generation
  - Reduce time identifying traits and number of progeny to maintain
  - Phenotype at earlier developmental stages
  - Segregating populations – 539 F2 progenies
  - Germplasm – mini core, wilds, common breeding lines

- Empirically determined the phenotype of each ahFAD2 genotype
  - Statistical analysis
  - Genetics

---

**ahFAD2**

- ahFAD2 genotyping
  - G448A in ahFAD2A
  - Ains442 in ahFAD2B

- Frequency
  - 36% aa
  - 64% AA
  - 100% BB