Genetics Approaches to Enhance Provitamin A and Total Carotenoids in Maize Grain

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2007 Corn Breeders’ School

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Overview

- What are Carotenoids? Why are they Important?
- Total Carotenoids - Synthetic Populations
- Provitamin A - A619 x SC55 Population
- Conclusions
What are Carotenoids?

- Naturally-Occurring Pigments
  - Coloration of Flowers, Birds, Insects, Fruits, Vegetables, etc.

- Over 600 Identified in Nature
  - Four Significant in Maize: Lutein, Zeaxanthin, β-Carotene, β-Cryptoxanthin

- Two Main Functions in Plant Cells
  - Accessory Pigment for Light Harvesting
  - Provide Protection Against Oxidative Damage
Carotenoid Biosynthetic Pathway

- GGPP
- Phytoene
- Phytofluene
- \( \zeta \) - carotene
- Neurosporene
- Lycopene
- \( \beta \) - LCY
- \( \epsilon \) - LCY
- \( \alpha \) - carotene
- \( \beta \) - carotene
- \( \beta \) - cryptoxanthin
- Lutein
- Zeaxanthin
Provitamin A Carotenoids

- Vitamin A
- β-carotene
- Vitamin A
- OH
- Vitamin A
- β-cryptoxanthin
- Vitamin A
- α-carotene
Health Benefits - Carotenoids

- Antioxidant Effects
- Reduced Risk of Macular Degeneration & Cataracts
  - US Population Deficient in Xanthophylls
- Possible Inhibitors of Certain Cancer Types
- Maintains Health Immune, Respiratory, Gastrointestinal Systems
Health Benefits – Vitamin A

- Deficiencies Lead to Xerophthalmia, Blindness, and Premature Death
- VAD = Leading Cause of Preventable Blindness in Children
- Annual Mortality in Children from VAD is over 1.2 Million
- VAD Most Negatively Affects Children and Pregnant/Nursing Women
Important for Animal Feed

- Yellow Corn is Main Source of Provitamin A in Swine & Poultry Diets
- Usually Supplemented w/ Synthetic or Natural Carotenoids - marigolds, alfalfa, etc.

Xanthophylls Essential in Poultry Diet - Coloration of Egg Yolks, Fat & Skin

- Xanthophylls
- Essential in Poultry Diet
- Coloration of Egg Yolks, Fat & Skin

[Image of two fried eggs in a pan]
Visual Selection in Synthetics

- Rank Intensity Orange Color; Need to Rank, not Score
- Starchy Crown is Problem When Ranking
- Visual Selection is Easy...Anyone Can Do It!
## Carotenoid Levels - Synthetics

(µg/ g)

<table>
<thead>
<tr>
<th>Synthetic Pedigree</th>
<th>Year</th>
<th>Lut</th>
<th>Zeax</th>
<th>Bcrypt</th>
<th>Bcar</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>KUI3, KUI11, KUI43, KUI2007</td>
<td>2006</td>
<td>8.51</td>
<td>30.48</td>
<td>2.78</td>
<td>6.78</td>
<td>48.55</td>
</tr>
<tr>
<td>KUI3, KUI11, KUI43, KUI2007</td>
<td>2005</td>
<td>4.58</td>
<td>32.59</td>
<td>2.14</td>
<td>4.63</td>
<td>41.80</td>
</tr>
<tr>
<td>Fla949, PI186, SAPhoto, SAPhoto876Trop, Fla949161-2</td>
<td>2005</td>
<td>6.71</td>
<td>19.61</td>
<td>3.20</td>
<td>3.12</td>
<td>29.44</td>
</tr>
<tr>
<td>KUI3, KUI11, KUI43, KUI2007, SC55</td>
<td>2005</td>
<td>5.72</td>
<td>15.14</td>
<td>2.11</td>
<td>3.59</td>
<td>24.45</td>
</tr>
<tr>
<td>Illini Orange</td>
<td>2005</td>
<td>2.59</td>
<td>9.52</td>
<td>1.94</td>
<td>2.85</td>
<td>14.96</td>
</tr>
</tbody>
</table>
Previous QTL Analysis Suggests Candidate Genes

A632 x W64A F2:3s     IHOxB73 BC1:S1s

Y1 = Phytoene Synthase

ZDS = Zeta Carotene Desaturase

EC = Epsilon Cyclase

PS, ZDS, EC are in Carotenoid Biosynthetic Pathway
Previously Mapped QTL for Levels of Carotenoids to Regions with:

- Lycopene Epsilon Cyclase – Very Strongly Supported by Association Analysis
- Phytoene Synthase (Preliminary Support AA)
- Zeta Carotene Desaturase (Supported AA)

Ed Buckler and Carlos Harjes

May Represent Variation in Enzyme Activity
* QTL Also Goes to Regions That Do Not Have Biosynthetic Genes

* May Identify Suppressors or Enhancers of Genes in Pathway Or Upstream of Pathway, Precursors

* Could Be Used to Modify Flux Into and Within Pathway
SC55 Contains Alleles That Can Affect Flux in Pathway - Higher $\beta$-Carotene
A619xSC55 Mapping Population

Complements Previous Work

**A619**: Yellow Inbred, High Total Carotenoids

**SC55**: Light Yellow Inbred, High Proportion of $\beta$-Carotene

\[
\begin{align*}
F_1 & \quad \downarrow \text{self} \\
F_2 \text{ ear} & \quad \downarrow \text{self} \\
F_{2:3} \text{ population} & \\
& (227 \text{ individuals}) \\
& \text{Sib-Mated}
\end{align*}
\]
Phenotypic Analysis

- $F_{2:3}$ Population Ranked from Darkest Orange to Lightest Yellow, #1-227
- $F_{2:3}$ also Scored for Overall Color and Segregation
- Sib-mated $F_3$ Seed Sent for HPLC Analysis
Statistical Analysis

- **JoinMap 3.0** - Evaluate Marker Data, Create Linkage Map
  - 104 Markers Currently in Map
  - Need to Fill in Gaps & Increase Resolution

- **PLABQTL Software for Composite Interval Mapping (CIM) Analysis**
  - 1000 Permutations done to Determine Critical LOD Scores
# CIM Analysis - Visual Traits

<table>
<thead>
<tr>
<th>Trait</th>
<th>Bin Position</th>
<th>QTL Position</th>
<th>Support Interval</th>
<th>LOD Effect</th>
<th>Dom Effect</th>
<th>Add Effect</th>
<th>Partial R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rank</td>
<td>6.01</td>
<td>48</td>
<td>38-50</td>
<td>3.16</td>
<td>1.557</td>
<td>-11.424</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>9.07</td>
<td>248</td>
<td>244-248</td>
<td>19.48</td>
<td>15.566</td>
<td>65.502</td>
<td>35.7</td>
</tr>
</tbody>
</table>

\[ R^{2a} = 49.1\% \]
<table>
<thead>
<tr>
<th>Trait</th>
<th>Bin</th>
<th>QTL Position</th>
<th>Suppport Interval</th>
<th>LOD</th>
<th>Dom Effect</th>
<th>Add Effect</th>
<th>Partial R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>9.02</td>
<td>44</td>
<td>38-52</td>
<td>4.66</td>
<td>-0.71</td>
<td>-0.247</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>9.07</td>
<td>162</td>
<td>148-172</td>
<td>4.79</td>
<td>0.113</td>
<td>0.521</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>9.07</td>
<td>248</td>
<td>244-248</td>
<td>20.41</td>
<td>30.695</td>
<td>1.578</td>
<td>36.1</td>
</tr>
</tbody>
</table>

\[ R^2a = 56.2\% \]
Combined QTL

Rank & Color

Chromosome 6

Chromosome 9

- Rank
- Color
- Both
Candidate Gene

- White cap1 (wc1) Maps to Bin 9.07-9.08
- Cleavage Enzyme Involved in Carotenoid Degradation Process
- Tandem Copies Present in Mutant - Select Those with Least Copies to Reduce Degradation
## CIM Analysis for HPLC Data

### Individual Compounds

<table>
<thead>
<tr>
<th>Trait</th>
<th># QTL</th>
<th>Adj $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutein</td>
<td>4</td>
<td>53.2%</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>5</td>
<td>47.3%</td>
</tr>
<tr>
<td>Zeinoxanthin</td>
<td>5</td>
<td>27.9%</td>
</tr>
<tr>
<td>β-cryptoxanthin</td>
<td>4</td>
<td>40.1%</td>
</tr>
<tr>
<td>α-carotene</td>
<td>1</td>
<td>4.8%</td>
</tr>
<tr>
<td>Trans- β -carotene</td>
<td>3</td>
<td>13.9%</td>
</tr>
<tr>
<td>9-cis- β -carotene</td>
<td>2</td>
<td>25.1%</td>
</tr>
<tr>
<td>13-cis- β -carotene</td>
<td>4</td>
<td>16.9%</td>
</tr>
<tr>
<td>15-cis- β -carotene</td>
<td>1</td>
<td>1.9%</td>
</tr>
<tr>
<td>Phytoene</td>
<td>4</td>
<td>38.6%</td>
</tr>
<tr>
<td>Phytofluene</td>
<td>3</td>
<td>38.8%</td>
</tr>
</tbody>
</table>
## CIM Analysis for HPLC Data

### Totals and Ratios

<table>
<thead>
<tr>
<th>Trait</th>
<th># QTL</th>
<th>Adj R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total B-carotene</td>
<td>4</td>
<td>15.9%</td>
</tr>
<tr>
<td>Total colored compounds</td>
<td>5</td>
<td>49.4%</td>
</tr>
<tr>
<td>Total carotenoids</td>
<td>2</td>
<td>53.4%</td>
</tr>
<tr>
<td>Colorless / colored</td>
<td>3</td>
<td>23.9%</td>
</tr>
<tr>
<td>α-car / β-car branches</td>
<td>3</td>
<td>38.2%</td>
</tr>
<tr>
<td>β-car / β-crypt</td>
<td>3</td>
<td>36.5%</td>
</tr>
<tr>
<td>β-crypt / Zeax</td>
<td>2</td>
<td>31.8%</td>
</tr>
<tr>
<td>β-car + β-crypt / Zeax</td>
<td>3</td>
<td>32.4%</td>
</tr>
<tr>
<td>α-car / Lutein</td>
<td>1</td>
<td>25.6%</td>
</tr>
<tr>
<td>ProA / Xanthophyll</td>
<td>3</td>
<td>34.1%</td>
</tr>
</tbody>
</table>
Important QTL at Same Location in Bin 9.07/08 Using Both Visual and HPLC Data

- **Candidate Gene, white cap1**

- Bin 7.02 – Zeta-Carotene Desaturase Previously Mapped to this Region

- Bin 10.05 – Possible Hydroxylase gene or Gene Influencing Hydroxylase Affecting Flux in B-Carotene Branch

- Overall Strategy Increase Flux into Pathway, Slow Flux into A-Carotene Branch, Slow Degradation
Carotenoid Biosynthetic Pathway

GGPP → Phytoene → Phytofluene → ζ-carotene → Neurosporene → Lycopene

β-LCY → ε-LCY

*α-carotene *β-carotene *β-cryptoxanthin

Lutein Zeaxanthin
Marketplace Study

- Gave 3 Types of Maize Porridge (Xhima)
  - White, IL & Mozambique
  - Orange, IL

- Collected Demographic & Sensory Perception Data

- Gave 1kg Bag of White Maize Meal, Offered to Trade for Varying Amount of Orange Meal or Tomatoes
Acknowledgements

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- Dr. Elizabeth Johnson Lab
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- Dr. Alex Winter-Nelson
- Chandra Paul
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