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

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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

PSY
y1

GGPP

Phytoene

PDS
vp5

Phytofluene

ζ- carotene

ZDS
vp9

Neurosporene

Lycopene

β- LCY
ε- LCY

β- LCY

* α- carotene

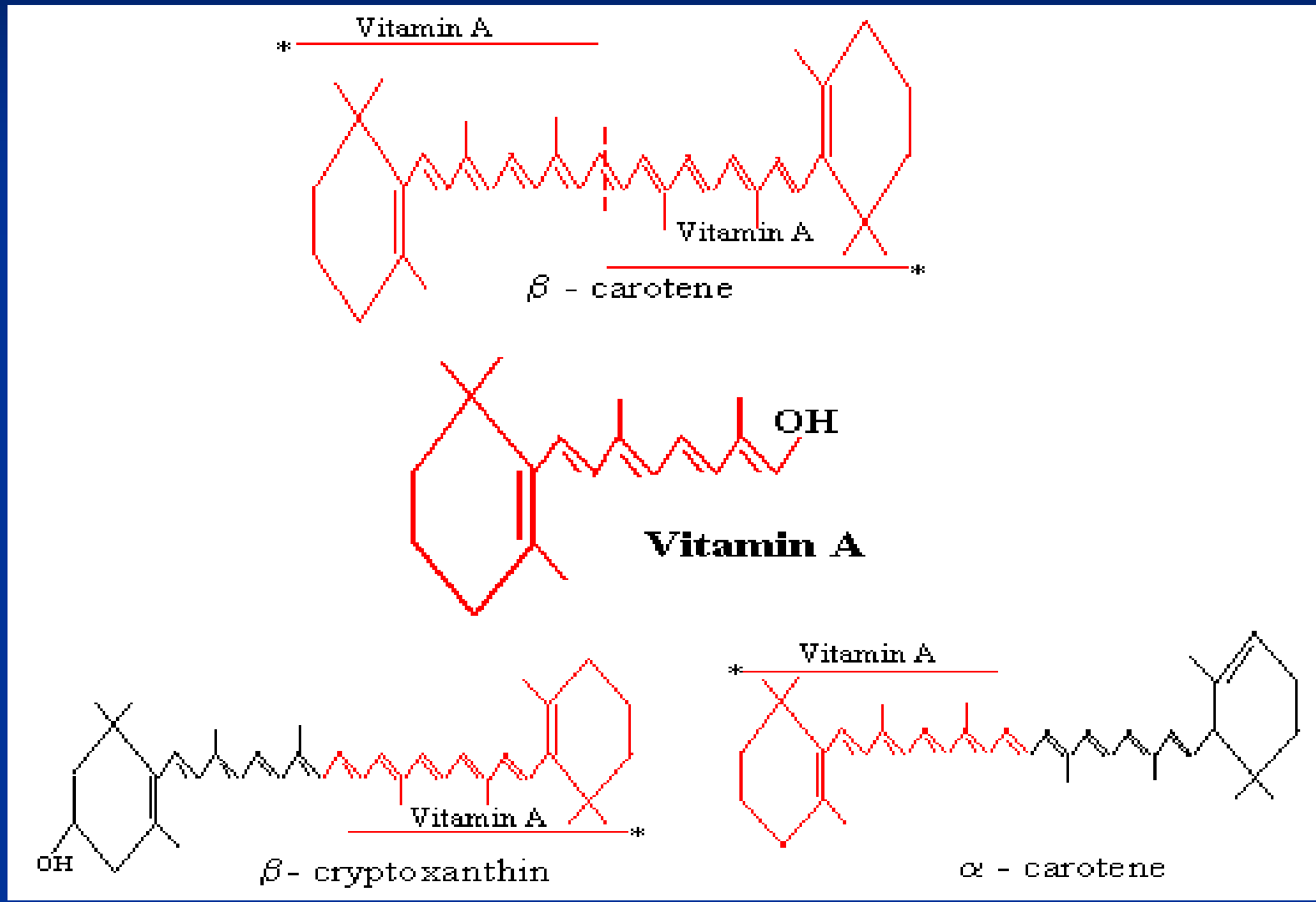
β- carotene *

* β-cryptoxanthin

Lutein

Zeaxanthin

Provitamin A Carotenoids



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

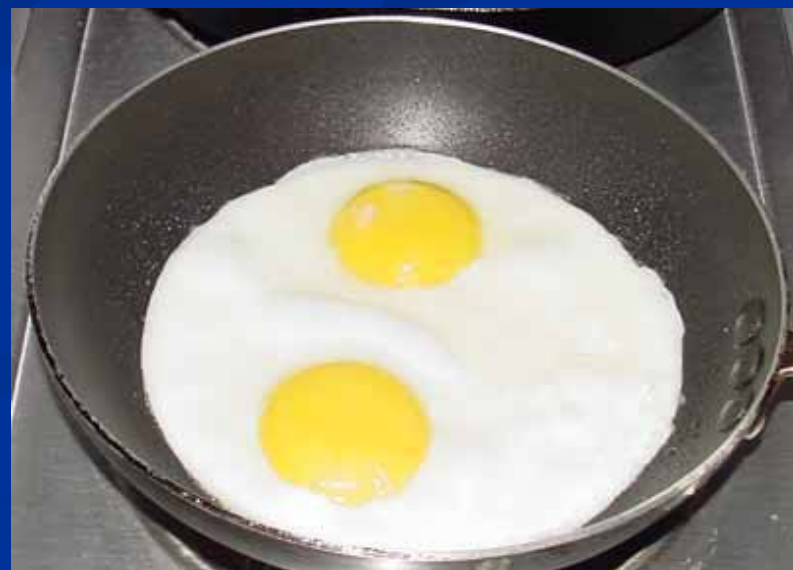
Health Benefits – Vitamin A

- 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.**

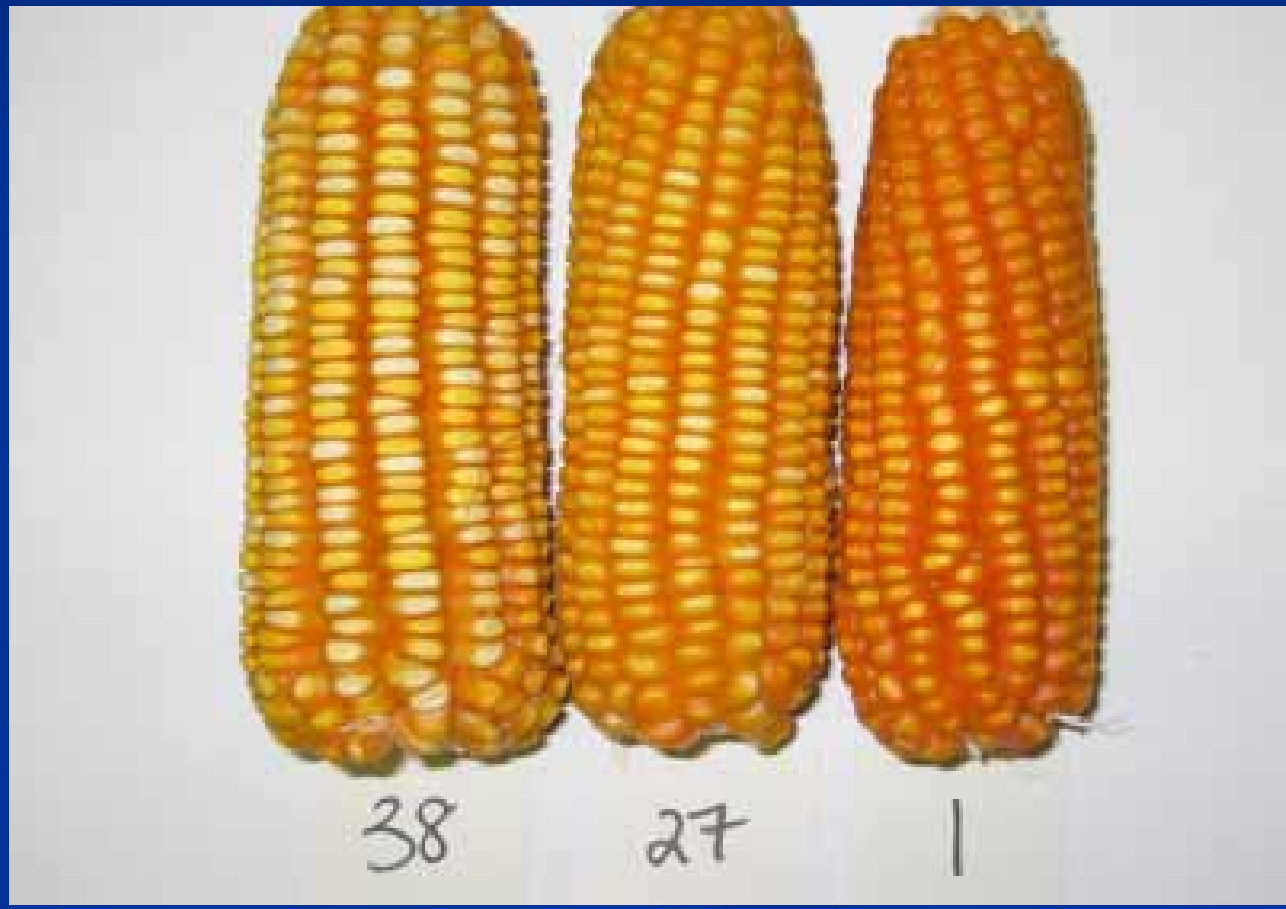
Xanthophylls Essential in Poultry Diet - Coloration of Egg Yolks, Fat & Skin




Lambert

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

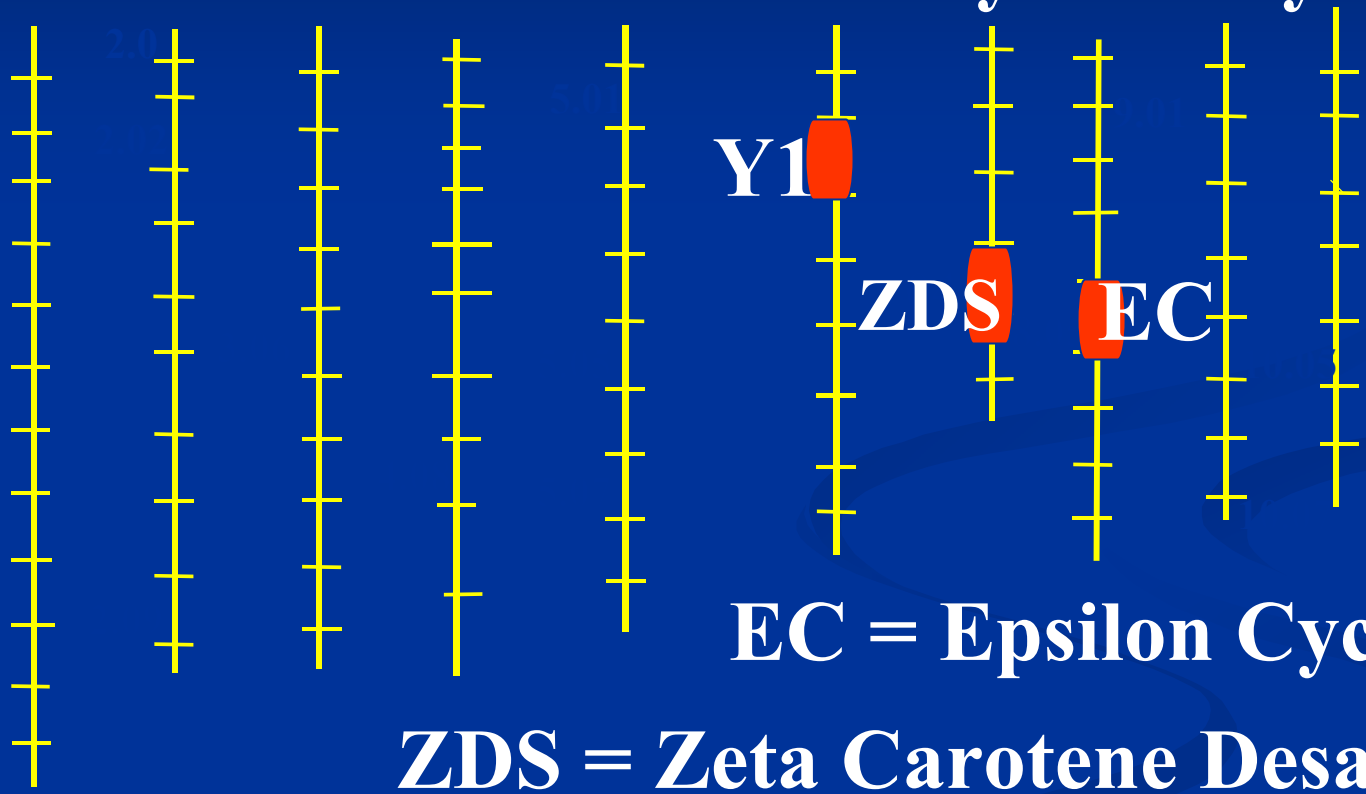
(ug/g)

Synthetic Pedigree	Year	Lut	Zeax	Bcrypt	Bcar	Total
KUI3, KUI11, KUI43, KUI2007	2006	8.51	30.48	2.78	6.78	48.55
KUI3, KUI11, KUI43, KUI2007	2005	4.58	32.59	2.14	4.63	41.80
Fla949, PI186, SAPhoto, SAPhoto876Trop, Fla949161-2	2005	6.71	19.61	3.20	3.12	29.44
KUI3, KUI11, KUI43, KUI2007, SC55	2005	5.72	15.14	2.11	3.59	24.45
Illini Orange	2005	2.59	9.52	1.94	2.85	14.96

Previous QTL Analysis Suggests Candidate Genes

A632 x W64A F2:3s IHOxB73 BC1:S1s

Y1 = Phytoene Synthase



EC = Epsilon Cyclase

ZDS = Zeta Carotene Desaturase

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 Assocation Analysis

Phytoene Synthase (Preliminary Support AA),

Zeta Carotene Desaturase (Supported AA)
Ed Buckler and Carlos Harjes

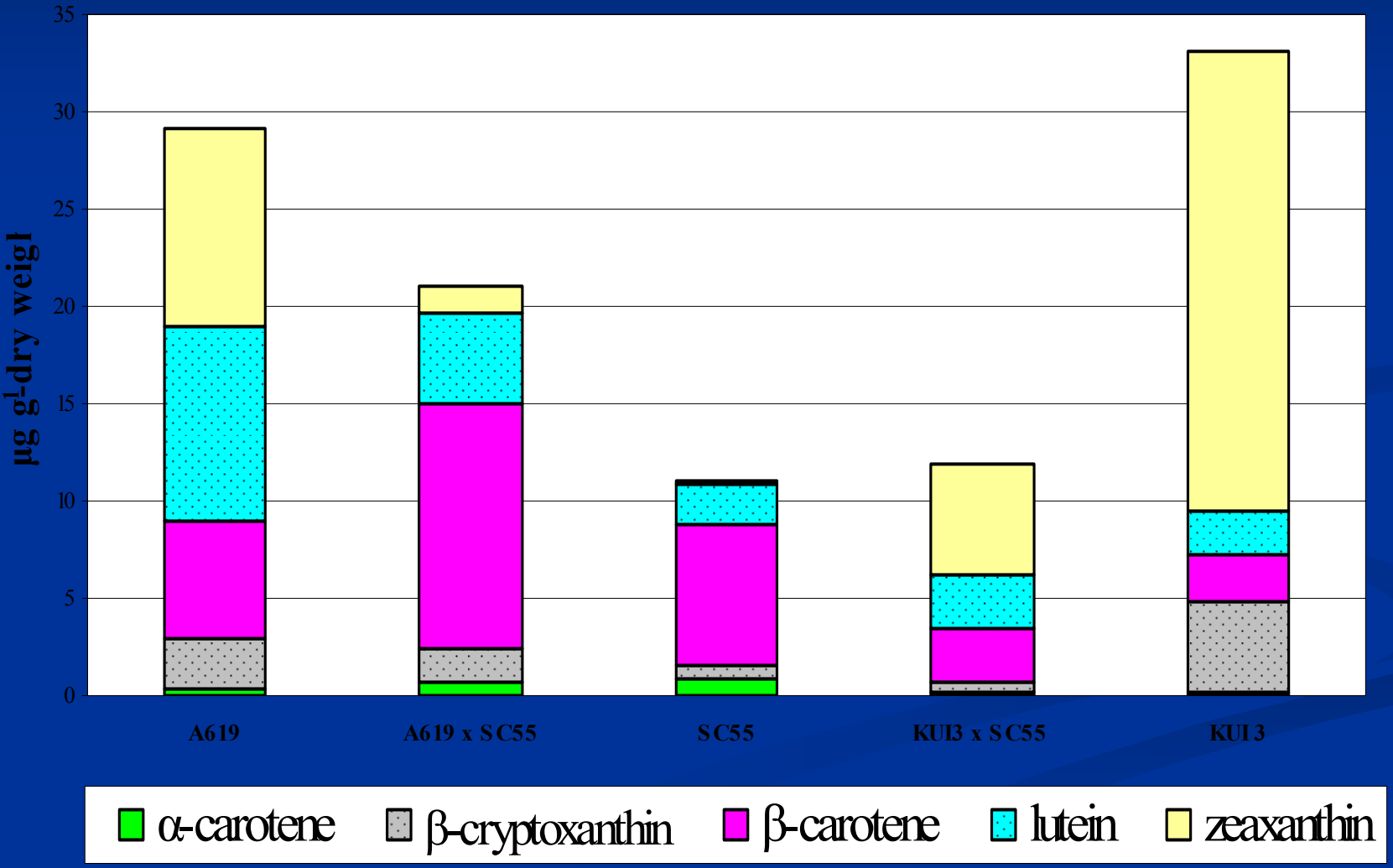
May Represent Variation in Enzyme Activity

* 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 β-Carotene



A619xSC55 Mapping Population Complements Previous Work

A619: Yellow Inbred, High Total Carotenoids

SC55: Light Yellow Inbred, High Proportion of β-Carotene

F_1

↓ self

F_2 ear

↓ self

$F_{2:3}$ population

(227 individuals)

Sib-Mated

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 Criticial LOD Scores**

CIM Analysis - Visual Traits

Trait	Bin	QTL Position	Suppport Interval	LOD	Dom Effect	Add Effect	Partial R^2
Rank	**6.01**	**48**	**38-50**	**3.16**	**1.557**	**-11.424**	**6.6**
	9.07	**248**	**244-248**	**19.48**	**15.566**	**65.502**	**35.7**

R^{2a} = 49.1%

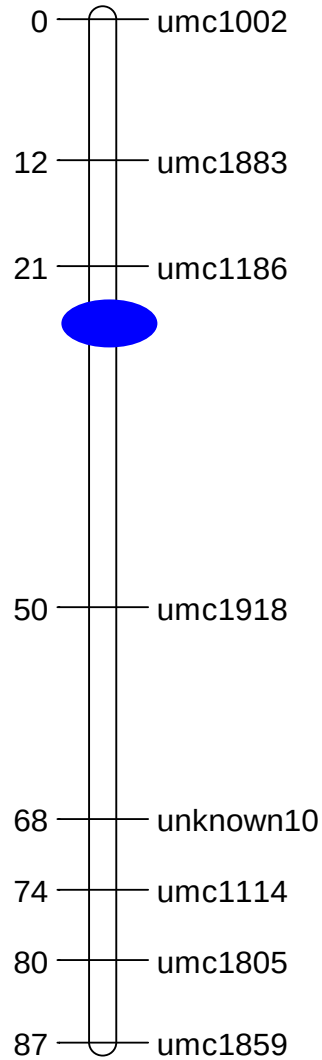
CIM Analysis - Visual Traits

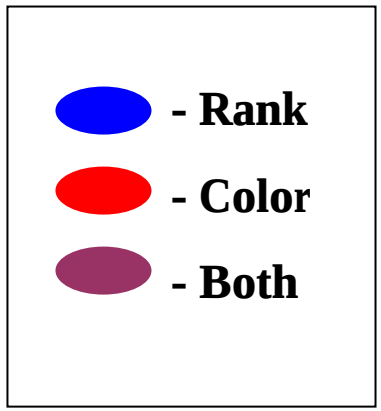
Trait	Bin	QTL Position	Suppport Interval	LOD	Dom Effect	Add Effect	Partial R^2
Color	9.02	44	38-52	4.66	-0.71	-0.247	9.6
	9.07	162	148-172	4.79	0.113	0.521	9.4
	9.07	248	244-248	20.41	30.695	1.578	36.1

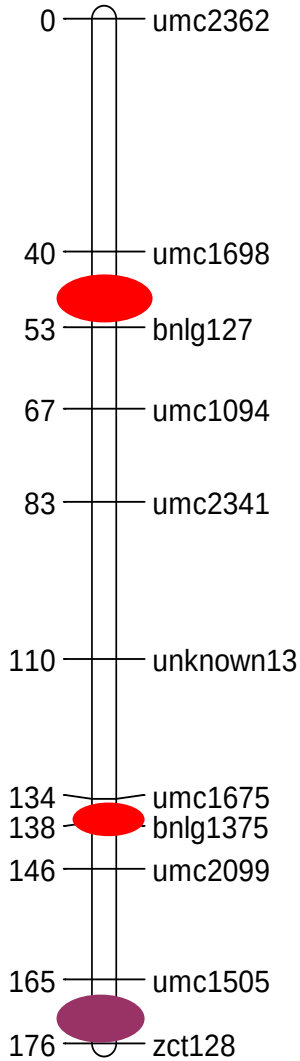
R^{2a} = 56.2%

Combined QTL

Rank & Color







Chromosome 6

Chromosome 9

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

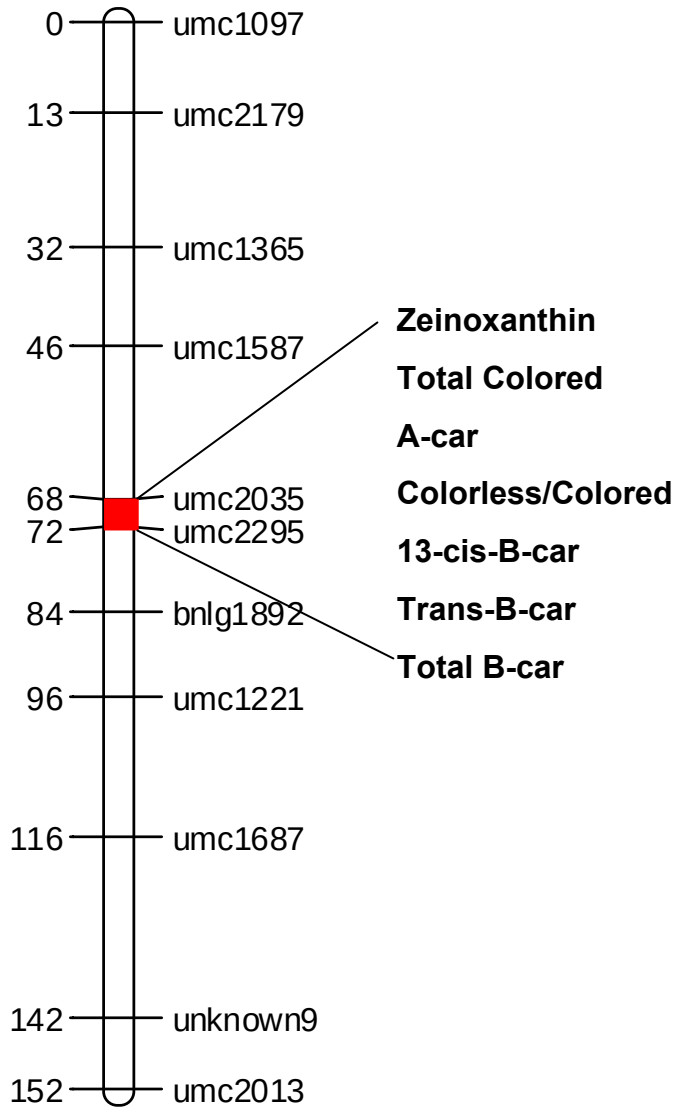
Individual Compounds

Trait	# QTL	Adj R^2
Lutein	4	53.2%
Zeaxanthin	5	47.3%
Zeinoxanthin	5	27.9%
β-cryptoxanthin	4	40.1%
α-carotene	1	4.8%
Trans- β -carotene	3	13.9%
9-cis- β -carotene	2	25.1%
13-cis- β -carotene	4	16.9%
15-cis- β -carotene	1	1.9%
Phytoene	4	38.6%
Phytofluene	3	38.8%

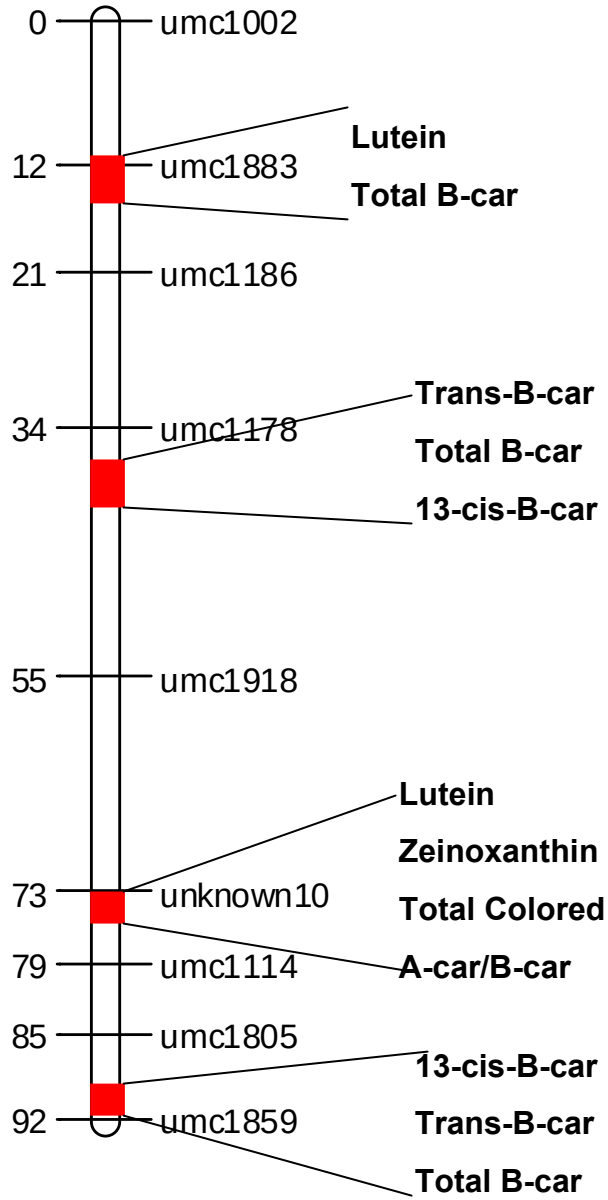
CIM Analysis for HPLC Data

Totals and Ratios

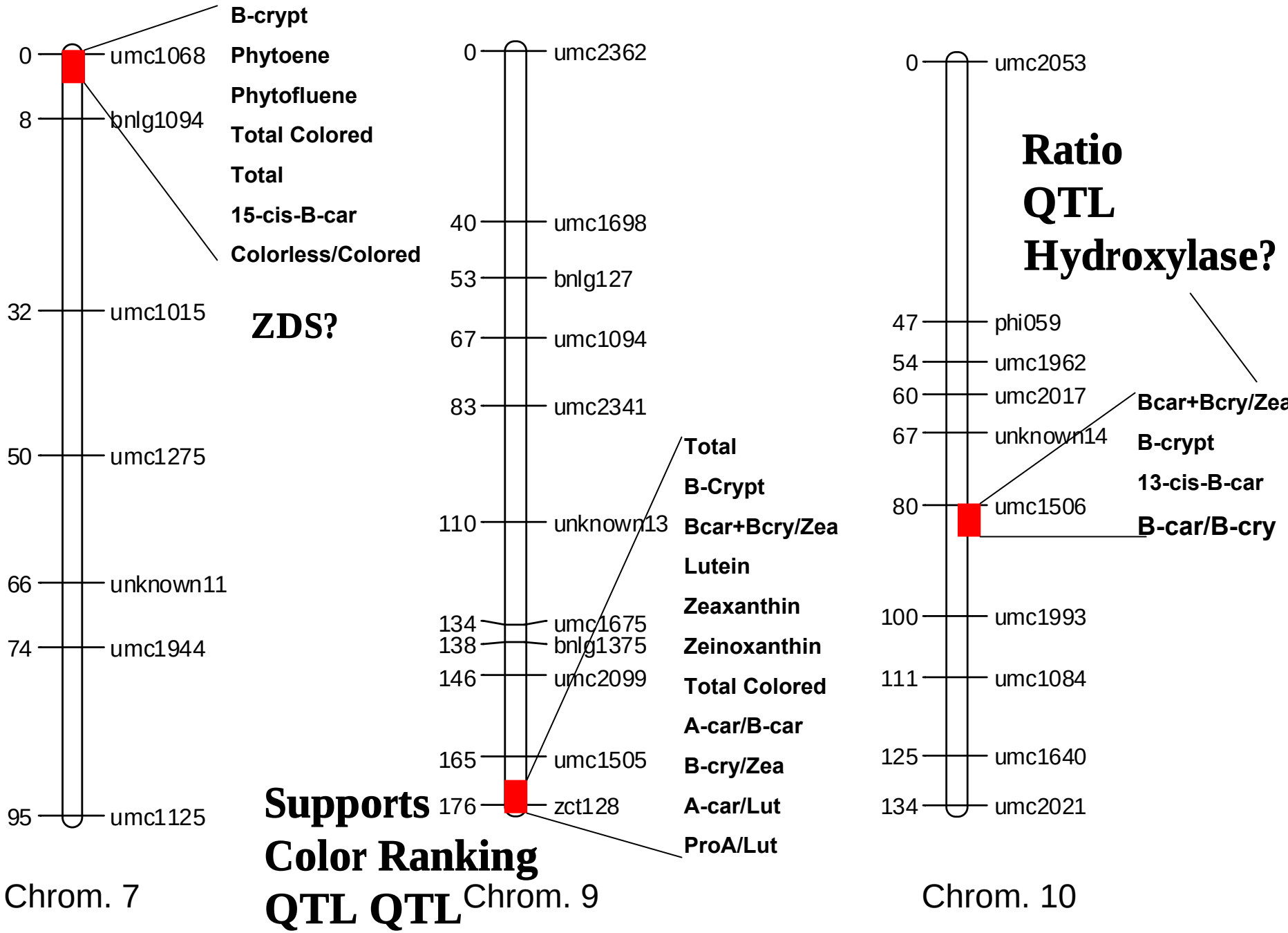
Trait	# QTL	Adj R^2
Total B-carotene	4	15.9%
Total colored compounds	5	49.4%
Total carotenoids	2	53.4%
Colorless / colored	3	23.9%
α-car / β-car branches	3	38.2%
β-car / β-crypt	3	36.5%
β-crypt / Zeax	2	31.8%
β-car + β-crypt / Zeax	3	32.4%
α-car / Lutein	1	25.6%
ProA / Xanthophyll	3	34.1%



Chrom. 5



Chrom. 6


0
umc1068
8
bnlg1094
32
umc1015
50
umc1275
66
unknown11
74
umc1944
95
umc1125
B-crypt
Phytoene
Phytofluene
Total Colored
Total
15-cis-B-car
Colorless/Colored
ZDS?
Chrom. 7
0
umc2362
40
umc1698
53
bnlg127
67
umc1094
83
umc2341
110
unknown13
134
umc1675
138
bnlg1375
146
umc2099
165
umc1505
176
zct128
Total
B-Crypt
Bcar+Bcry/Zea
Lutein
Zeaxanthin
Zeinoxanthin
Total Colored
A-car/B-car
B-cry/Zea
A-car/Lut
ProA/Lut
Supports Color Ranking QTL QTL
Chrom. 9
0
umc2053
47
phi059
54
umc1962
60
umc2017
67
unknown14
80
umc1506
100
umc1993
111
umc1084
125
umc1640
134
umc2021
Ratio QTL Hydroxylase?
Bcar+Bcry/Zea
B-crypt
13-cis-B-car
B-car/B-cry
Chrom. 10

- 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**

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Neurosporene

Lycopene

β- LCY
ε- LCY

β- 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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