The future will bring more vitamins in our food.

Vitamin A

Africa and Asia have most nutrient deficiency.

Pregnant women and children are most at risk worldwide.

Increasing global population.

Larger yields.

Rising global heat and drought.

Breeders can select for orange.

We can map for color!

Fly over fields to collect data.

Rapidly convert successful local varieties.

Increased automation is only going to get faster.

AI can help with data.

Wonderful opportunities for genomic selection.

Global vitamin enhancement of maize grain.

Prof. Tobert Rocheford
GLOBAL ENHANCEMENT OF VITAMIN AND NUTRIENT COMPOSITION OF GRAIN

OPPORTUNITIES FOR USING ROBOTICS, ARTIFICIAL INTELLIGENCE WITH GENOMIC SELECTION TO GREATLY ENHANCE:

GAIN FROM SELECTION TO BREED MORE NUTRITIOUS & HIGHER YIELDING CEREAL GRAINS FOR DEVELOPING & DEVELOPED WORLD

TORBERT ROCHEFORD
Dawn!
World Hunger and CIMMYT’s Presence in Maize

Degree of Food deprivation

- Low prevalence and low depth
- Moderate prevalence and low depth or low prevalence and moderate depth
- Moderate prevalence and moderate depth
- High prevalence and moderate depth or moderate prevalence and high depth
- High prevalence and high depth
- Not assessed countries with populations under 1 million or insufficient data

Source:
FIVIMS (Food insecurity and vulnerability information and mapping SOFI 2000 (State of Food Insecurity in the World)
http://www.fivims.net/
Vitamin A deficiencies, darker colors more severe.
Vitamin A deficiency can result in night blindness and increased susceptibility to infections and can eventually result in death (Combs 2012).

Estimated 250,000 to 500,000 children go blind every year as result of Vit A deficiency, and half of these die within one year of losing eyesight [www.who.int/nutrition/topics/vad/en/](http://www.who.int/nutrition/topics/vad/en/)

Prompted nutritional interventions - including promoting increased consumption of plant-based carotenoids by HarvestPlus maize biofortification program for Africa [www.harvestplus.org](http://www.harvestplus.org)
Biofortification

- Biofortification – the development of food crops that fortify themselves

  Bouis and Welch, 2010

- Steps to Success:

  Breeding
  Efficacy
  Adoption
Minor Difference in Structure, Molecule Does Not Fit Into Certain Sites, thus no Vitamin A activity. Requires HPLC Analysis to Differentiate. All carotenoids are Anti-oxidants, may protect from Free Radicals
Carotenoid Biosynthetic Pathway

**Synthase**
- GGPP
  - Phytoene
  - Phytofluene
  - ζ-carotene
- Neurosporene

**Cyclases**
- Lycopene
  - β-LCY
  - ε-LCY

**Hydroxylases**
- α-carotene!
- β-carotene!

**Desaturases**
- PSY
- PDS
- ZDS

**Hydroxylases**
- β-cryptoxanthin!
Carotenoid Deficiencies are Globally Prevalent

US: Age-related Macular Degeneration

Developing countries: Vitamin A Deficiency

Zeaxanthin
Lutein

40 yr
65 yr
75 yr

Prevalence
AMD
Macular Degeneration

- In the developed world, a more common problem is age-related macular degeneration.

- A diet rich in foods containing carotenoids may help.
World Population is Growing
Need to increase agricultural productivity at faster rate next fifty years than past fifty years (*Green Revolution*) to feed hungry people

<table>
<thead>
<tr>
<th>Region</th>
<th>Year</th>
<th>Rural Population</th>
<th>Urban Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>World</td>
<td>1960</td>
<td>2,011,140,059</td>
<td>1,013,708,223</td>
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<td>2014</td>
<td>3,341,185,000</td>
<td>3,838,295,000</td>
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<td></td>
<td>2050</td>
<td>3,100,355,800</td>
<td>6,355,642,000</td>
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<table>
<thead>
<tr>
<th>Region</th>
<th>Year</th>
<th>Rural Population</th>
<th>Urban Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>East-Asia and Pacific (developing countries only)</td>
<td>1960</td>
<td>745,558,073</td>
<td>151,788,265</td>
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<tr>
<td></td>
<td>2014</td>
<td>977,859,000</td>
<td>1,047,358,000</td>
</tr>
<tr>
<td></td>
<td>2050</td>
<td>591,072,000</td>
<td>1,582,536,000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region</th>
<th>Year</th>
<th>Rural Population</th>
<th>Urban Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-Saharan Africa (developing countries only)</td>
<td>1960</td>
<td>194,050,070</td>
<td>33,538,837</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>598,726,000</td>
<td>361,386,000</td>
</tr>
<tr>
<td></td>
<td>2050</td>
<td>941,922,000</td>
<td>1,204,353,000</td>
</tr>
</tbody>
</table>
Global Climate Change May Reduce Crop Productivity

Decreasing yields due to higher temperatures, water-stress.

Forecasted yield declines particularly strong in:
- South-Asia - 14% decline in rice production, 44-49% decline in wheat, 9-19% decline in maize
- Sub-Saharan Africa - projected declines by 2050 of 15%, 34% and 10% for rice, wheat and maize
- Projected yield losses higher developing countries
- We need research on heat and drought tolerance and grain yield to complement biofortification.
DNA Diversity Between Two Random Inbreds of Maize is Comparable to that Between Humans and Chimpanzees, Maize is Genetically Very Diverse. DNA Assay are Automatable - Robotics. Artificial Intelligence Could Contribute Significantly Also, These ears bring forward cultural acceptance issues for white versus yellow maize in Africa……
Africa Acceptance – Using Orange to Overcome Preference For White and Concerns about Yellow Grain. Orange Also Associated with More Total Carotenoids and More Flux into Carotenoid Pathway – More to Convert to ProVitaminA
Acknowledgments

- US Agency for International Development
- Major Goodman NC State / Ed Buckler USDA/ARS now Cornell Carlos Harjes
- Sultana Islam, Jeff Wong, Chandra Paul, Robyn Allscheid, Cathy Kandianis, Ines Carvahlo
- HarvestPlus, HP Maize Colleagues
- Patrick Schaub and Peter Beyer Lab
Breeders Can Select for Darker Orange Visually
Cheap, Easy, Low Tech, High Throughput

INVOLVE LOCAL BREEDERS IN DEVELOPING COUNTRIES

However, More Orange Does NOT Mean Much More
ProVitA Carotenoids
Carotenoid Biosynthetic Pathway

**Synthase**
- PSY (y1)
- GGPP → Phytoene → Phytofluene → ζ-carotene → Neurosporene

**Desaturases**
- PDS (vp5)
- ZDS (vp9)

**Cyclases**
- θ-LCY
- ε-LCY

**Hydroxylases**
- Lycopene → α-carotene! → β-carotene! → β-cryptoxanthin!
- Lutein → Zeaxanthin
Schematic Diagram - Maize Lycopene Epsilon Cyclase

Asteriks - Polymorphisms Significantly Associated with Changes in Flux between Lutein and Zeaxanthin Branches of Pathway.

White Triangles - 5’ transposable element insertions
Transcript Levels Correlate with insertions

Harjes, Rocheford, …Buckler  Science 119:330-333
ZmCrtR-B1 gene structure and significant polymorphisms

The fact that we have identified alleles of genes that are DNA Based means we have useful information for provitamin A breeding that can be automated,

Yan/Kandianis……Rocheford  Nature Genetics 42, 322–327 (2010)
Successfully Used Basic, Upstream Research and Results:

Visual Selection Dark Orange = More Flux into Pathway (Gas Pedal)

*Select Weakest Allele of Lycopene Epsilon Cyclase* – More Flux towards Beta – Branch (Steering Wheel)

*Select Weakest Allele of Beta Carotene Hydroxylase (crtRB1)* – More Beta – Carotene (Strong Brake)
Provitamin A Content of Maize Grain (Goodman-Buckler Diversity Lines)

- Commercial hybrids
- Orange Flints
- Advanced CIMMYT Conventional Selection Breeding Lines
- ASMAS Breeding Lines S₂ generation

MAS = Allele Specific Marker Assisted Selection
Selection for Dark Orange Visually and Weakest Alleles of Lycopene Epsilon Cyclase and Beta Carotene Hydroxylase (crtRB1) by molecular markers.....

.....resulted in RAPIDLY reaching and attaining target levels in 75% subtropical adapted breeding materials from HP/CIMMYT

.....HarvestPlus/CIMMYT then well surpassed target levels in breeding lines
<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Type germplasm/maturity</th>
<th>pVAC ug/gDW</th>
<th>CrtB1 favorable allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>KUI carotenoid syn-FS11-1-1-B-B-B-B-B-B-B-B-B-B</td>
<td>Sub-trop/Early</td>
<td>6.81</td>
<td></td>
</tr>
<tr>
<td><strong>(KUI carotenoid syn-FS11-1-1-B-B-B-B-B)</strong>/(KU1409/DE3/KU1409)S2-18-2-B-B-3(MAS:L4H1)-1-B-B-B</td>
<td>Sub-trop/Early</td>
<td><strong>35.72</strong></td>
<td>+</td>
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<tr>
<td>KUI carotenoid syn-FS17-3-2-B-B-B-B-B-B-B-B-B-B-B-B</td>
<td></td>
<td>6.72</td>
<td></td>
</tr>
<tr>
<td><strong>(KUI carotenoid syn-FS17-3-2-B-B-B-B)</strong>/(KU1409/DE3/KU1409)S2-18-2-B-B-1(MAS:L4H1)-5-B-B-B</td>
<td>Sub-trop/Early</td>
<td>29.02</td>
<td>+</td>
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<tr>
<td><strong>(KUI carotenoid syn-FS17-3-2-B-B)</strong>/(KU1409/DE3/KU1409)S2-18-2-B-B-4(MAS:L4H1)-2-B-B-B</td>
<td>Sub-trop/Early</td>
<td>32.56</td>
<td>+</td>
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<tr>
<td>CML297</td>
<td>Sub-trop/V Late</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td><strong>(CML297)</strong>/(KU1409/DE3/KU1409)S2-18-2-B-B-2(MAS:L4H1)-4-B-B-B</td>
<td>Subtropical/late</td>
<td>15.55</td>
<td>+</td>
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<tr>
<td>Florida A plus Syn-FS2-2-1-B-B-B-B-B-B-B-B</td>
<td>Sub-trop/Early</td>
<td>8.47</td>
<td></td>
</tr>
</tbody>
</table>
Could We Map QTL for VISUAL SCORES for Color ORANGE in Nested Association Mapping Population

Chandler/Lipka...Buckler, Rocheford, Gore. Crop Science 2013
### 2009 Joint Family Results

<table>
<thead>
<tr>
<th>Marker</th>
<th>Chr.</th>
<th>location (bp)</th>
<th>Type III SS/ Total SS</th>
<th>Adjusted R²</th>
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<tbody>
<tr>
<td>m909</td>
<td>8</td>
<td>137,480,768</td>
<td>0.1518</td>
<td></td>
</tr>
<tr>
<td>m696</td>
<td>6</td>
<td>80,534,129</td>
<td>0.0479</td>
<td></td>
</tr>
<tr>
<td>m227</td>
<td>2</td>
<td>49,160,699</td>
<td>0.0364</td>
<td></td>
</tr>
<tr>
<td>m1025</td>
<td>9</td>
<td>147,130,708</td>
<td>0.0350</td>
<td></td>
</tr>
<tr>
<td>m959</td>
<td>9</td>
<td>19,293,027- 19,293,212</td>
<td>0.0218</td>
<td></td>
</tr>
<tr>
<td>m1087</td>
<td>10</td>
<td>136,530,988</td>
<td>0.0180</td>
<td></td>
</tr>
<tr>
<td>m811</td>
<td>7</td>
<td>137,632,654</td>
<td>0.0165</td>
<td></td>
</tr>
<tr>
<td>m384</td>
<td>3</td>
<td>178,773,618</td>
<td>0.0121</td>
<td>0.5552</td>
</tr>
</tbody>
</table>

- **Near psy1** (Chr 6) (80,876,153-80,876,391)
- **Near LCYε** (Chr 8) (137,539,312-137,706,774)
- **Near wc1** (Chr 9) (147,753,266-147-753,542)
- **Near crtRB1** (Chr 10) (135-685,906-135,687,612)

**Answer:** YES!

**ZDS and ZEP significant in 2010**  **Six loci related to carotenoid pathway found.**
“Opportunity to **Rapidly** Convert White or Yellow Commercial Hybrids in Zimbabwe, Ethiopia, and most **All of Subsaharan Africa**, and Rest of World---With Allele Specific Marker Assisted Selection **of just six-eight loci*** & Visual Selection Dark Orange Color. **GENETICS** (Accepted)

-To create Dark Orange High ProVA Hybrids with Highly Competitive Grain Yields.....

Need to cross orange by white to bring in provitamin A carotenoids

You get segregation
Shown on next slide
We need orange and High beta carotene and beta cryptoxanthin for provitamin A – need to Select alleles of DNA

This can be automated
And scaled up today, much moreso in the future... robotics and AI Could help enormously
DNA Sequence Based Progress

• DNA Sequencing & genotyping more and more automated, robotics, faster, assays done in the field, so selections made sooner.

• Artificial intelligence with help with interpreting data and selecting plants higher in proVA

• High Performance Liquid Chromatography (HPLC) very labor intensive, robotics can automate.

• Yet, if we get the DNA sequence to predict well proVA carotenoid phenotype, we hardly will need to do HPLC…..
Further testing of allelic selection in breeding populations

CIMMYT-Illinois High Total Carotenoid Populations

Temperate/ Tropical QTL Donors Diversity Panel

Single Kernel Genotyping for selected populations
Acknowledgments

BI-NATIONAL AGRICULTURAL RESEARCH AND DEVELOPMENT FUND
USAID
HARVEST PLUS
NATIONAL SCIENCE FOUNDATION
PIONEER HI BRED / DUPONT

CMMYT KEVIN PIXLEY, NATALIA PALACIOS, THANDA DWYLALO
IITA – ABEBBE MENKIR
ARS & CORNELL - ED BUCKLER, MIKE GORE

JEFF WONG          SULTANA ISLAM
ROBIN STEVENS      CATHY KANDIANIS
DEBRA SKINNER      JIANBING YAN
TYLER TIEDE        BRENDA OWENS
MEGAN FENTON

AND MANY, MANY OTHER GOOD SOULS….& INSTITUTIONS
Unmanned Aerial Vehicles

- Fly Over Crop Fields with Cameras and Sensors
- Provide Useful Spectral Information
- Hopefully Can be used in Selections and Management for Higher Grain Yields
- Programs of Katy Rainey, Keith Cherkauer, Melba Crawford and a number of postdocs, grad students, support scientists, pilots
Data Collection:
Assaying Canopy Coverage
6400 plots
7 dates
44,000 images

SigmaSCAN
Automated Image Analysis
Canopy Development and Precision Phenotyping

(Scale 1 to 5)
I guess this plot has CC 3

(Scale 1 to 100)
I am sure it is 43.208...

In future, robots will collect this data, more efficient, higher throughput, and humans can spend more time analyzing the data using artificial intelligence, and designing and growing bigger and better experiments.
Canopy Development and Precision Phenotyping

In future, maybe Robots will help Fly UAVs and Supercomputers With Artificial Intelligence will Analyze and Interpret Results…
Grad Students and Postdocs have real intelligence, not artificial – very powerful, well usually........
Dynamic Communicative Human Intelligence
Thermal Imagery

- Calibrate imagery based on target temp
As you can see, not even the sky is the limit

- Automation of DNA Sequencing, faster and faster, mind boggling
- Artificial Intelligence to help select hundreds, maybe thousands of genes at same time
- Faster Development More Nutritious Higher Yielding Grain Crops – We Need This!