the FUTURE will BRING
MORE VITAMINS in our
FOOD

AFRICA and
ASIA HAVE
MOST
NUTRIENT
DEFICIENCY

MOVE FROM CAPSULES

**VITAMIN
A**

I'M NOT
GMO

BREEDERS
can SELECT
for
ORANGE

WE CAN
MAP
for COLOR!

FLY OVER
FIELDS to
COLLECT DATA

RAPIDLY
CONVERT
SUCCESSFUL
LOCAL
VARIETIES

AI

CAN HELP WITH

data

INCREASED
Automation

GOING to GET **FASTER**

it's ONLY

PREGNANT
WOMEN
and
CHILDREN
are MOST AT RISK
WORLDWIDE

RISING
GLOBAL
HEAT &
DROUGHT

INCREASING
GLOBAL
POPULATION

LARGER
YIELDS

in the U.S.
MACULAR
DEGENERATION
an ISSUE

**GLOBAL VITAMIN
ENHANCEMENT**

OF MAIZE GRAIN

PROF. TOBERT ROCHEFORD

WONDERFUL OPPORTUNITIES
for GENOMIC SELECTION

541-49
SI7a-294-1

05-40740
FLRB
WCO

05-40740
FLRB
WCO

05-40740
FLRB
WCO

05-40740
FLRB
WCO

541-49
ma Chile

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

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/

Prompted nutritional interventions - including promoting increased consumption of plant-based carotenoids by HarvestPlus maize biofortification program for Africa www.harvestplus.org

Biofortification

- Biofortification – the development of food crops that fortify themselves

Bouis and Welch, 2010

- Steps to Success:

Breeding
Efficacy
Adoption

Chemical Structure of Pro-Vitamin A and Vitamin A

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

PSY
y1

GGPP

↓
Phytoene

↓
Phytofluene

↓
 ζ -carotene

↓
Neurosporene

Desaturases

β -LCY
 ϵ -LCY

α -carotene!

Lutein

Hydroxylases

Lycopene

Cyclases

β -LCY
 β -LCY

β -carotene!

β -cryptoxanthin!

Zeaxanthin

Carotenoid Deficiencies are Globally Prevalent

US: Age-related
Macular Degeneration

Zeaxanthin
Lutein

Developing countries:
Vitamin A Deficiency

Macular Degeneration

- In the developed world, a more common problem is age-related macular degeneration.
- A diet rich in foods containing carotenoids may help.

Normal vision

The same scene as viewed by a person with age-related macular degeneration

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

World

1960: 2,011,140,059 (rural); 1,013,708,223 (urban)

2014: 3,341,185,000 (rural); 3,838,295,000 (urban)

2050: 3,100,355,800 (rural); 6,355,642,000 (urban)

East-Asia and Pacific (developing countries only)

1960: 745,558,073 (rural); 151,788,265 (urban)

2014: 977,859,000 (rural); 1,047,358,000 (urban)

2050: 591,072,000 (rural); 1,582,536,000 (urban)

Sub-Saharan Africa (developing countries only)

1960: 194,050,070 (rural); 33,538,837 (urban)

2014: 598,726,000 (rural); 361,386,000 (urban)

2050: 941,922,000 (rural); 1,204,353,000 (urban)

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 Assays 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

**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

β -LCY
 ϵ -LCY

α -carotene!

Lutein

Hydroxylases

Lycopene

Cyclases

β -LCY
 β -LCY

β -carotene!

β -cryptoxanthin!

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

5' UTR variation

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

ASMAS Breeding
Lines S₂ generation

Advanced CIMMYT Conventional
Selection Breeding Lines

*!
22

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

Pedigree	Type germplasm/maturity	pVAC ug/gDW	CrtB1 favorable allele
KUI carotenoid syn-FS11-1-1-B-B-B-B-B-B-B-B-B	Sub-trop/Early	6.81	
(KUI carotenoid syn-FS11-1-1-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-3(MAS:L4H1)-1-B-B-B	Sub-trop/Early	35.72	+
KUI carotenoid syn-FS17-3-2-B-B-B-B-B-B		6.72	
(KUI carotenoid syn-FS17-3-2-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-1(MAS:L4H1)-5-B-	Sub-trop/Early	29.02	+
(KUI carotenoid syn-FS17-3-2-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-4(MAS:L4H1)-2-B-	Sub-trop/Early	32.56	+
CML297	Sub-trop/V Late	6.3	
(CML297/(KU1409/DE3/KU1409)S2-18-2-B)-B-2(MAS:L4H1)-4-B-B-B	Subtropical/late	15.55	+
Florida A plus Syn-FS2-2-1-B-B-B-B-B-B	Sub-trop/Early	8.47	
(Florida A plus Syn-FS2-2-1-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-3(MAS:L4H1)-2-B-	Sub-trop/Early	12.72	+

Could We Map QTL for VISUAL SCORES for Color ORANGE ? in Nested Association Mapping Population

Chandler/Lipka...Buckler, Rocheford, Gore. Crop Science 2013

Answer:
YES!

2009 Joint Family Results

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

★ 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)

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.....
- Will Require Effective Public – Private Sector Partnerships to do ***Rapidly***. **High Throughput Robotic Technologies will help.**” T.Rocheford. HarvestPlus Maize Conference, Zambia, 3/2013.

**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 more so 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

X

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 DWYIALO

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 temperature

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!