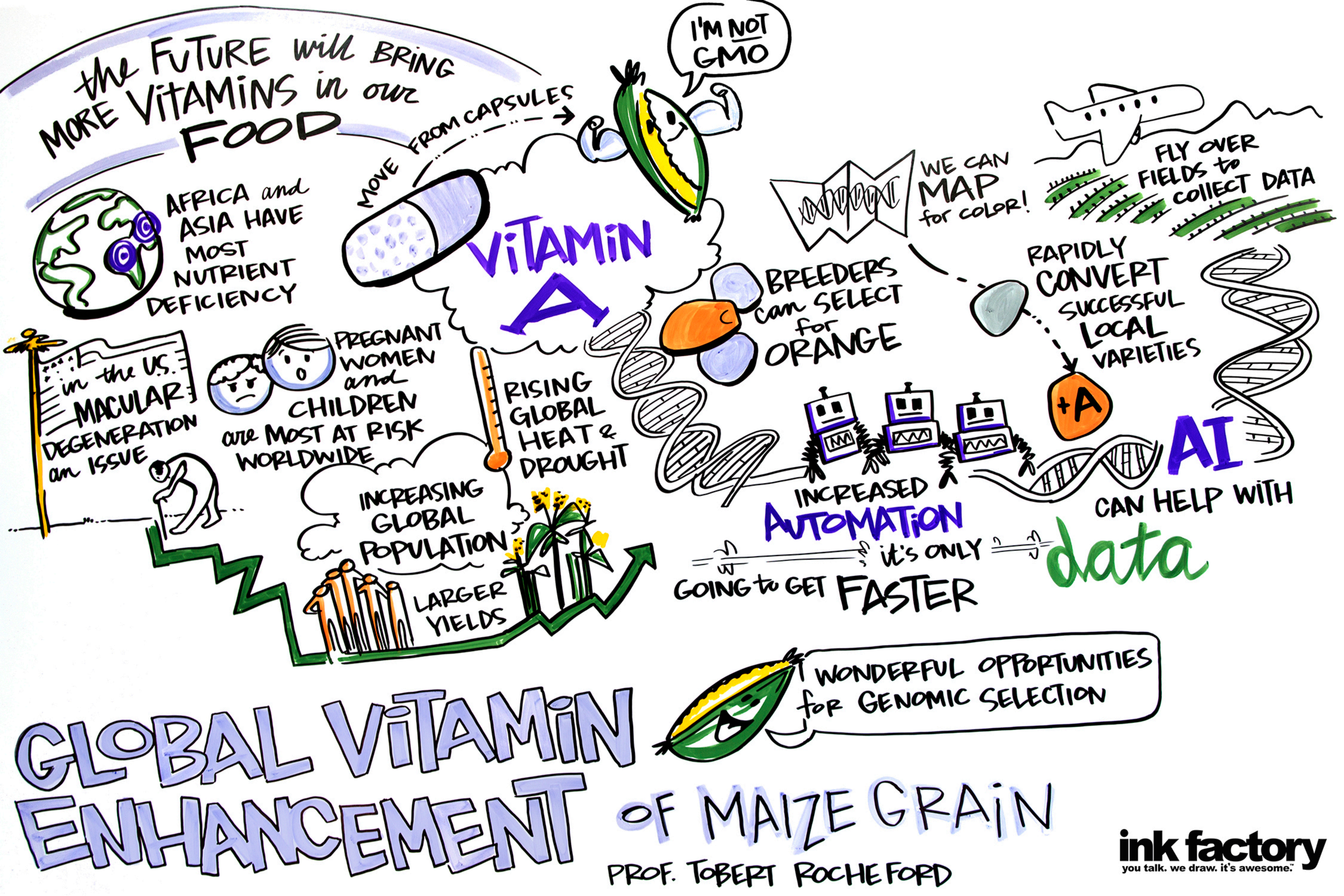


ink factory
you talk. we draw. it's awesome.™


541-49
541-49

GLOBAL ENHANCEMENT OF VITAMIN AND NUTRIENT COMPOSITION OF GRAIN

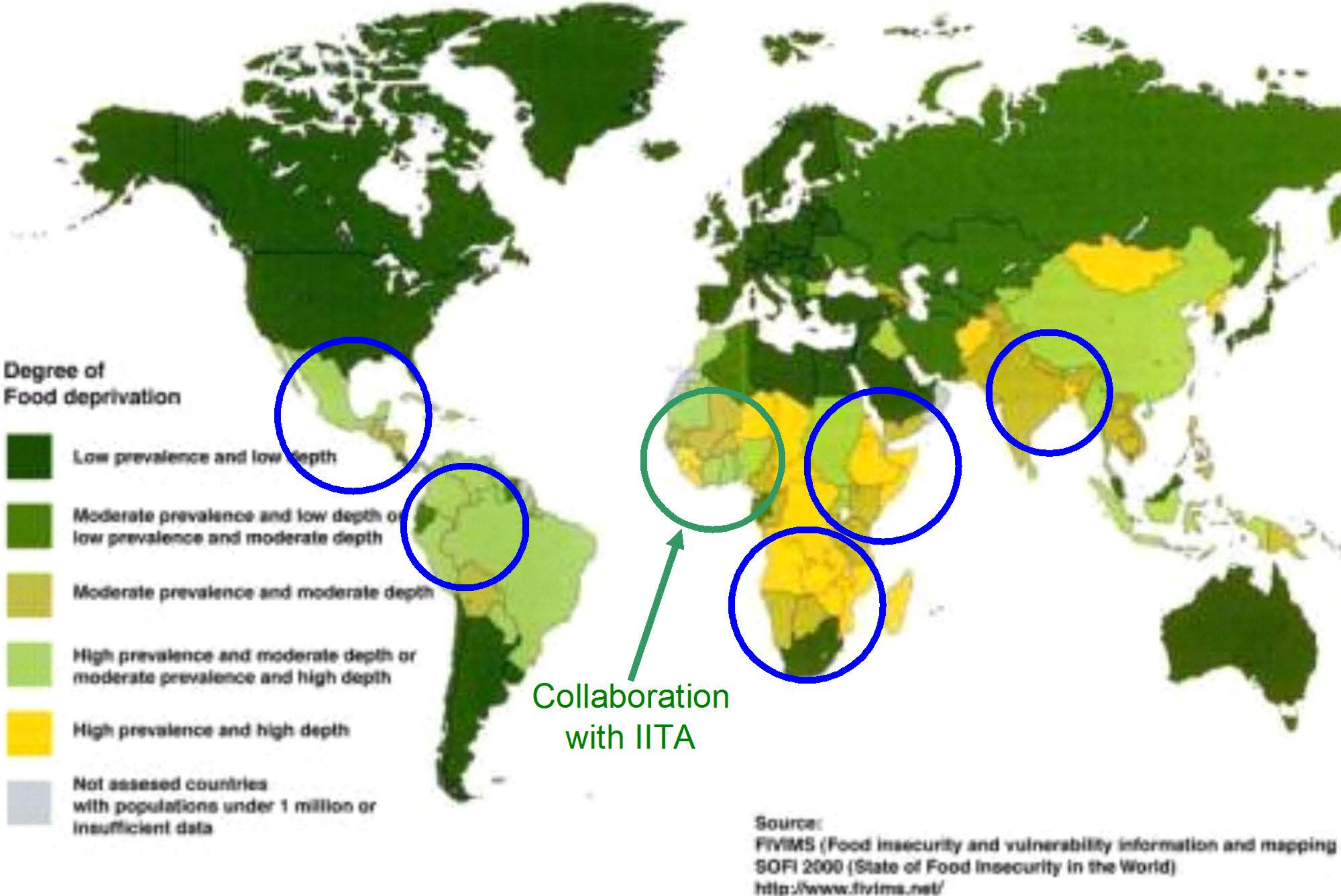
OPPORTUNITIES FOR USING ROBOTICS, ARTIFICIAL INTELLIGENCE WITH GENOMIC SELECTION TO GREATLY ENHANCE:

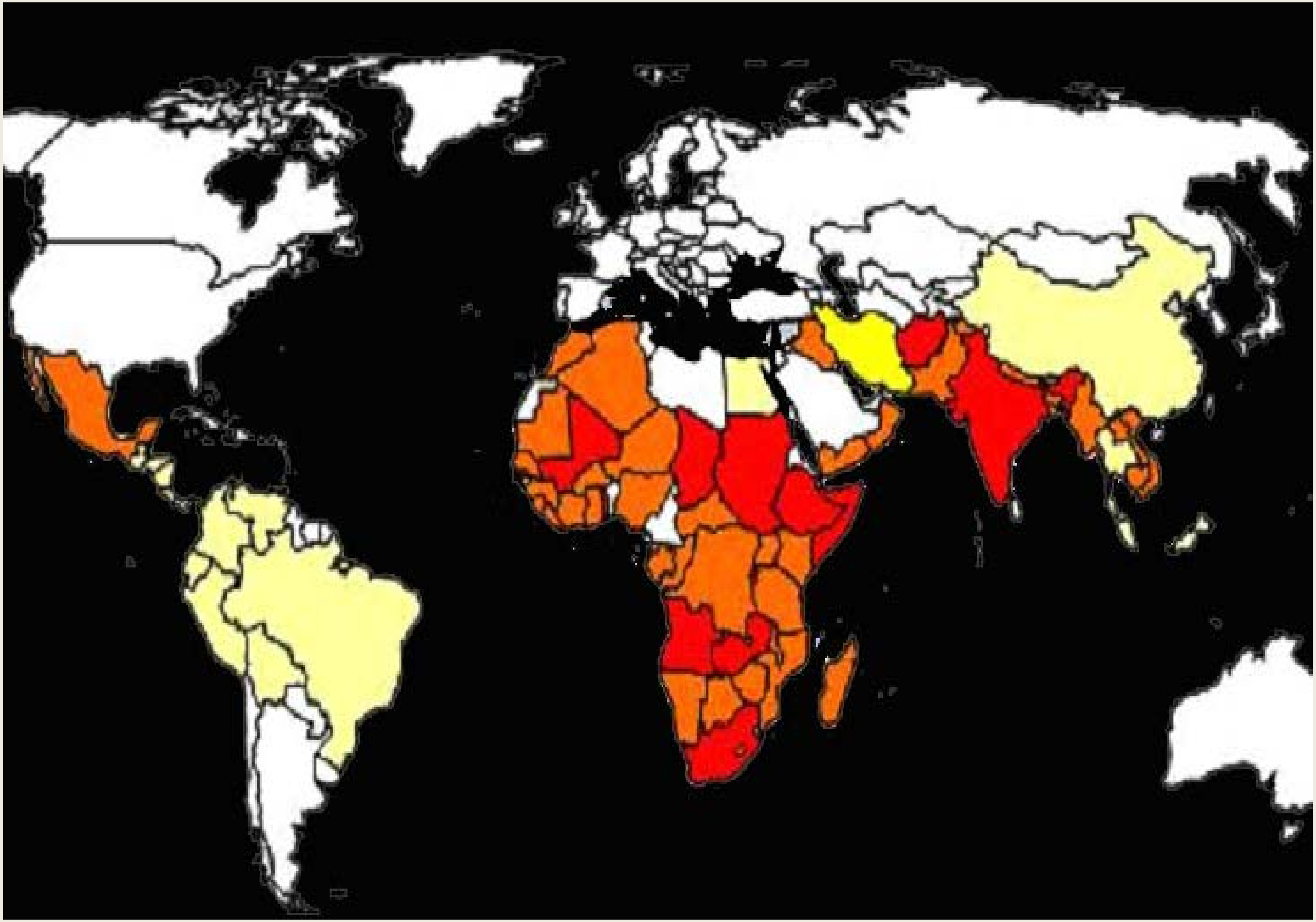
GAIN FROM SELECTION TO BREED MORE NUTRITIOUS & HIGHER YIELDING CEREAL GRAINS FOR DEVELOPING & DEVELOPED WORLD

TORBERT ROCHEFORD


Dawn!

World Hunger and CIMMYT's Presence in Maize





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

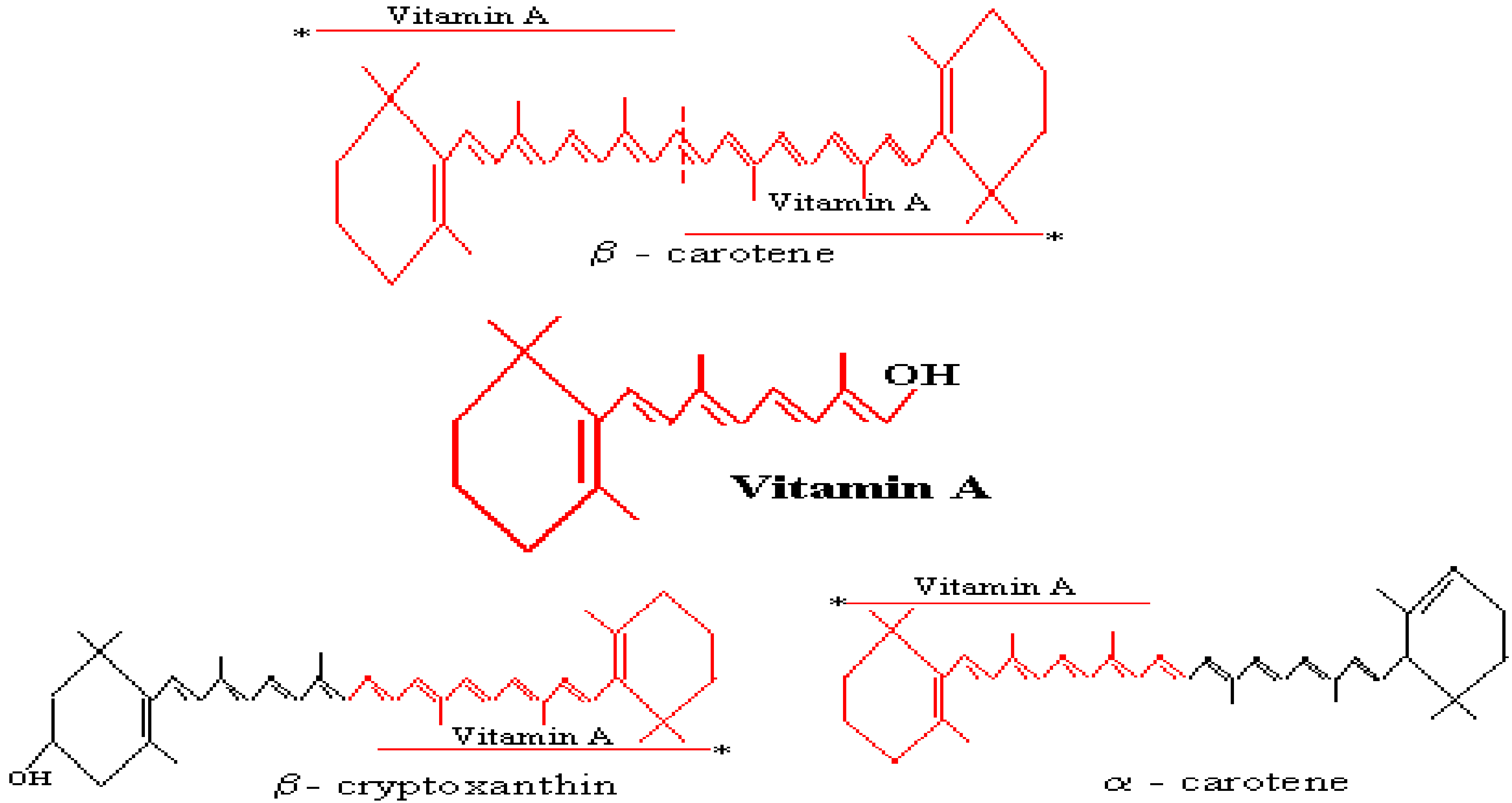
Bouis and Welch, 2010

- Steps to Success:

  Breeding
  Efficacy
  Adoption

T. Tiede

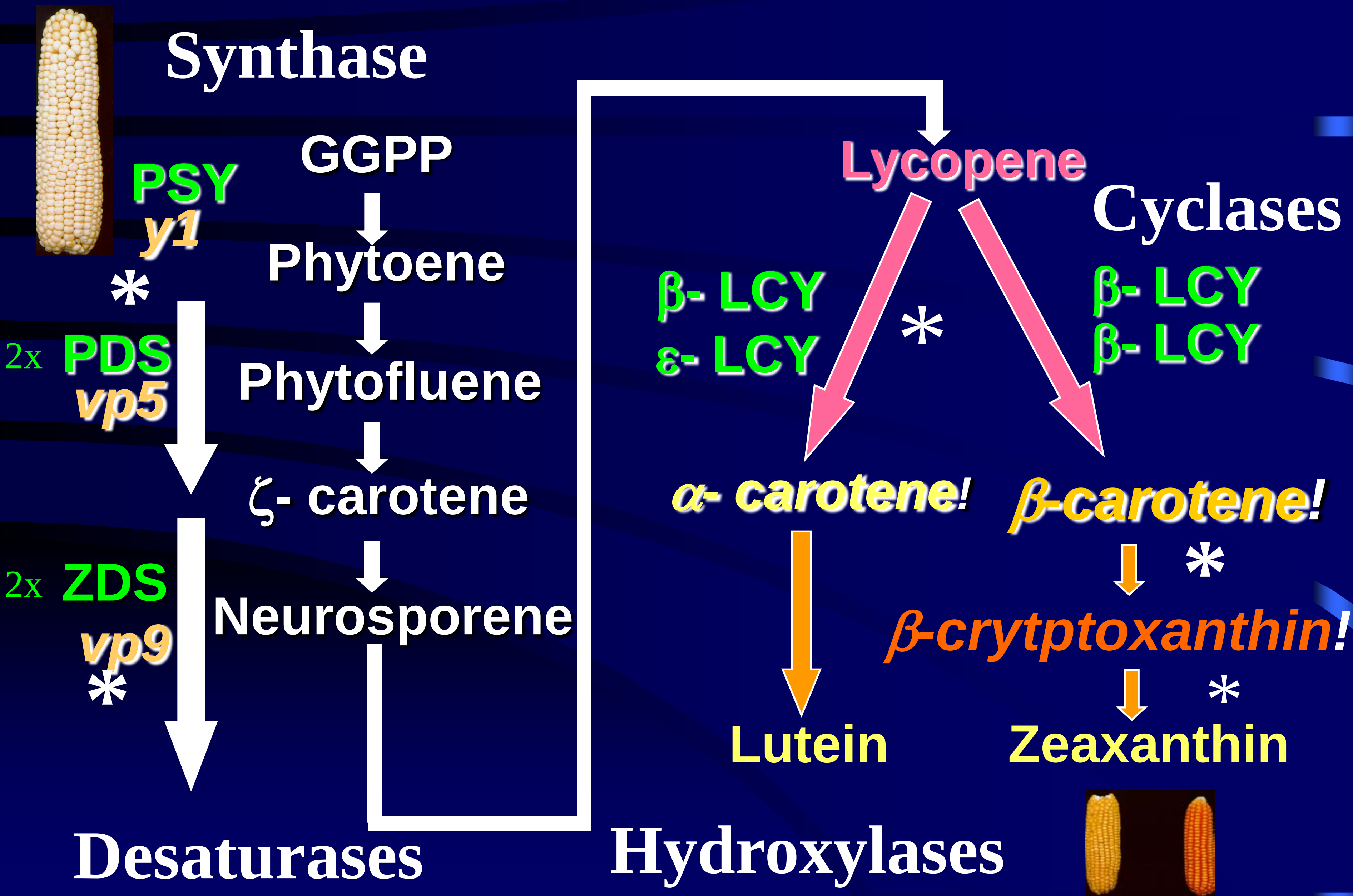
Chemical Structure of Pro-Vitamin A and Vitamin A



Minor Difference in Structure, Molecule Does Not Fit Into Certain Sites, thus no Vitamin A activity. Requires HPLC Analysis to Differentiate.

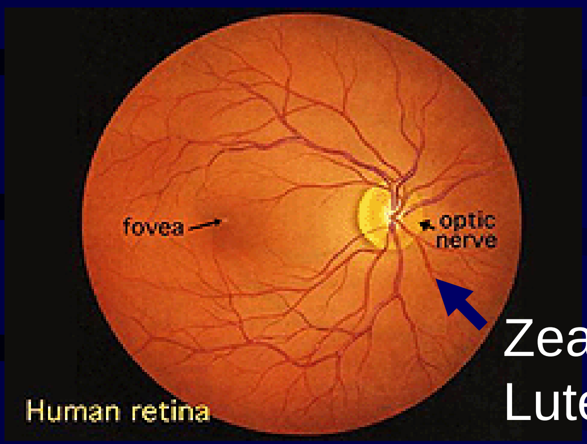
All carotenoids are Anti-oxidants, may protect from Free Radicals

Carotenoid Biosynthetic Pathway

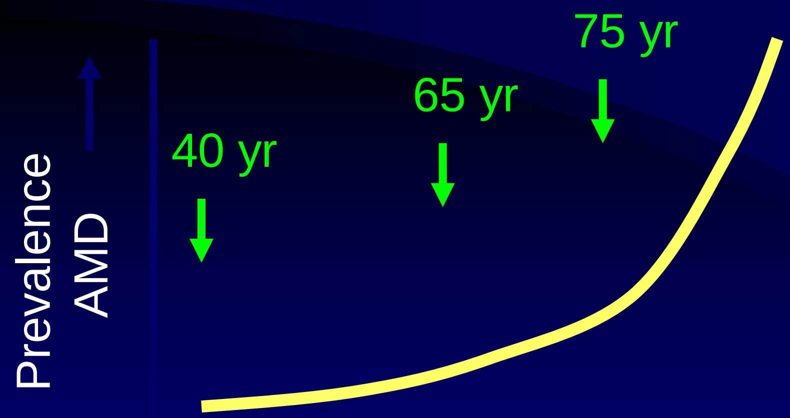


Carotenoid Deficiencies are Globally Prevalent

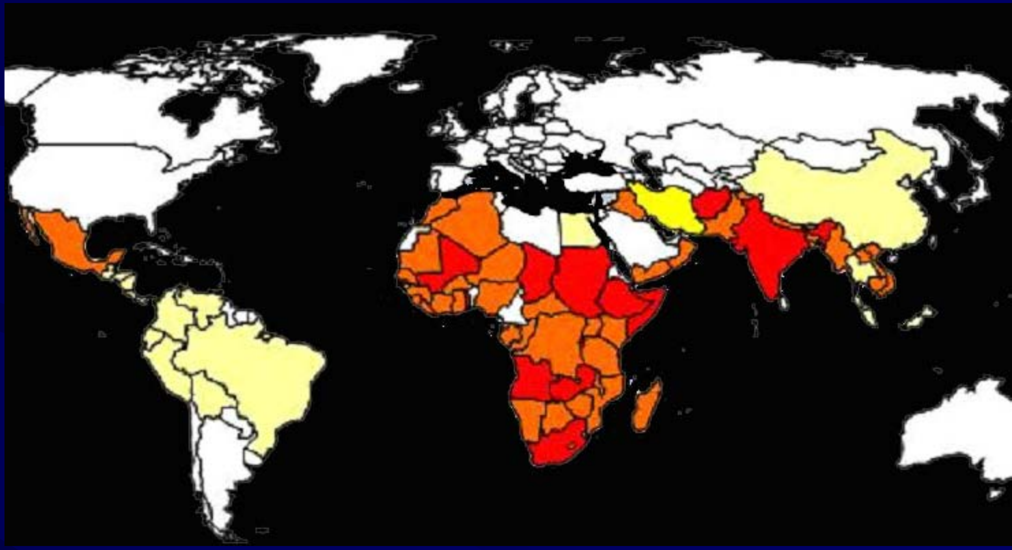
US: Age-related Macular Degeneration



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

National Eye Institute

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 Assay are Automatable - Robotics. Artificial Intelligence Could Contribute Significantly

Also, These ears bring forward cultural acceptance issues for white versus yellow maize in Africa……

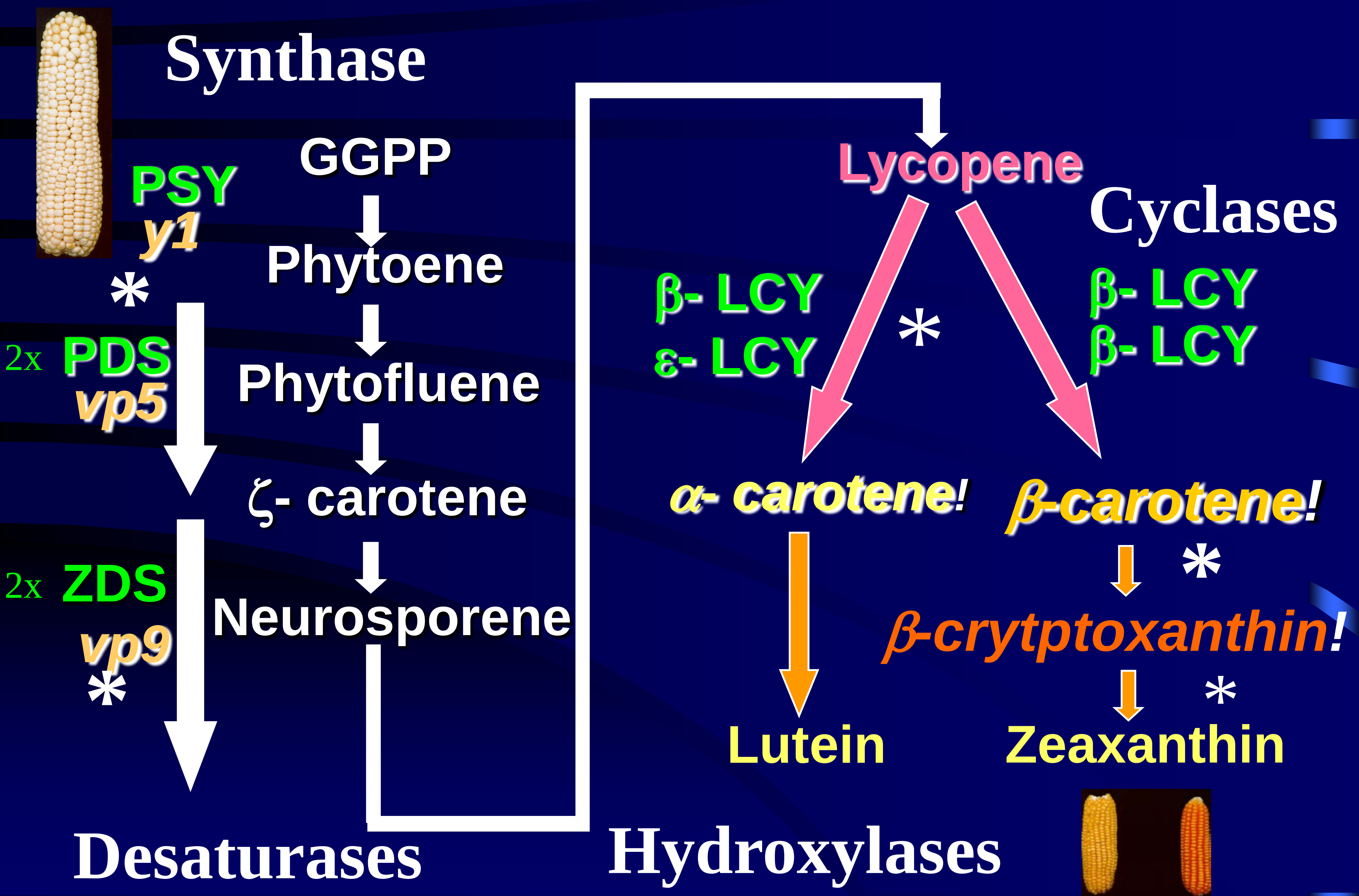
Africa Acceptance – Using Orange to Overcome Preference For White and Concerns about Yellow Grain. Orange Also Associated with More Total Carotenoids and More Flux into Carotenoid Pathway – More to Convert to ProVitaminA

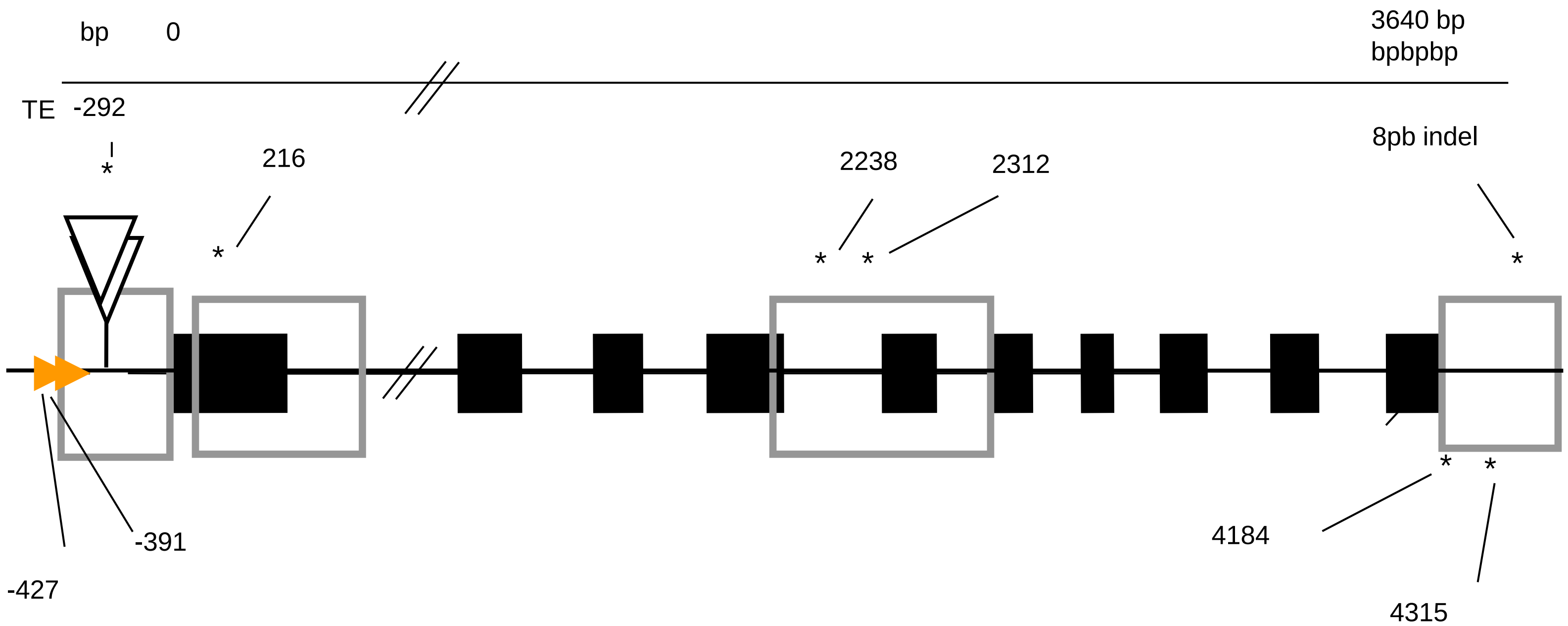
Breeders Can Select for Darker Orange Visually
Cheap, Easy, Low Tech, High Throughput

INVOLVE LOCAL BREEDERS IN DEVELOPING COUNTRIES

However, More Orange Does NOT Mean Much More ProVitA Carotenoids

Carotenoid Biosynthetic Pathway





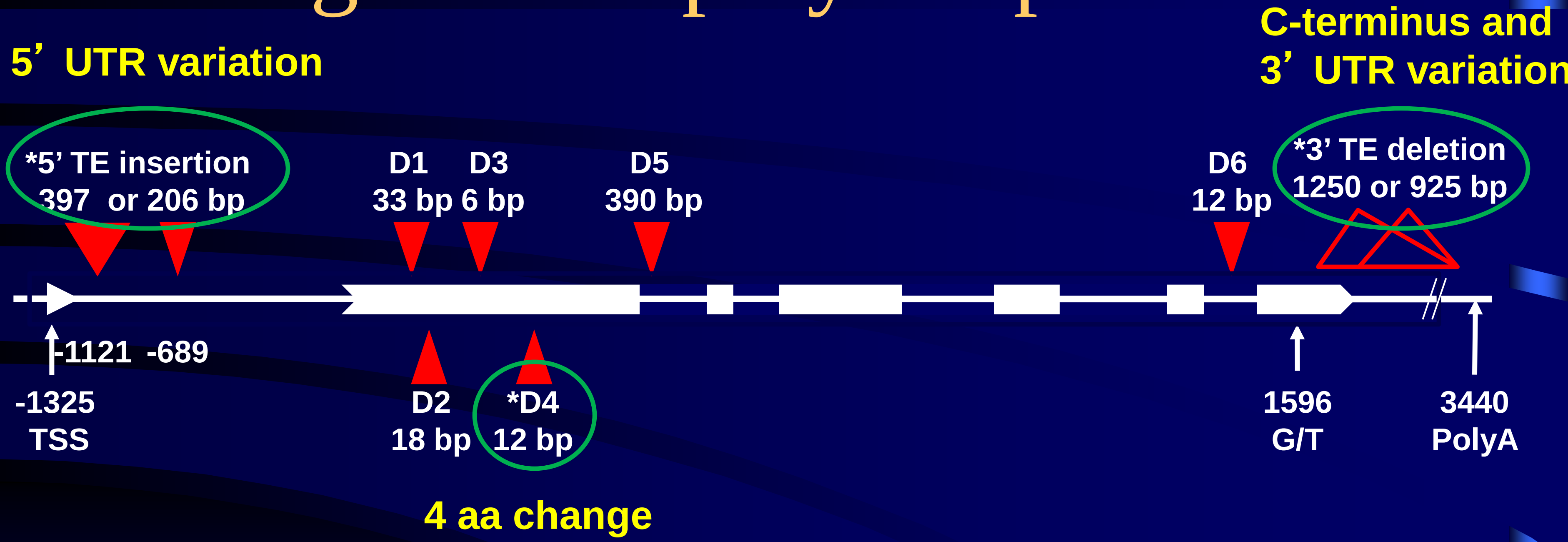
Schematic Diagram - Maize Lycopene Epsilon Cyclase

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,

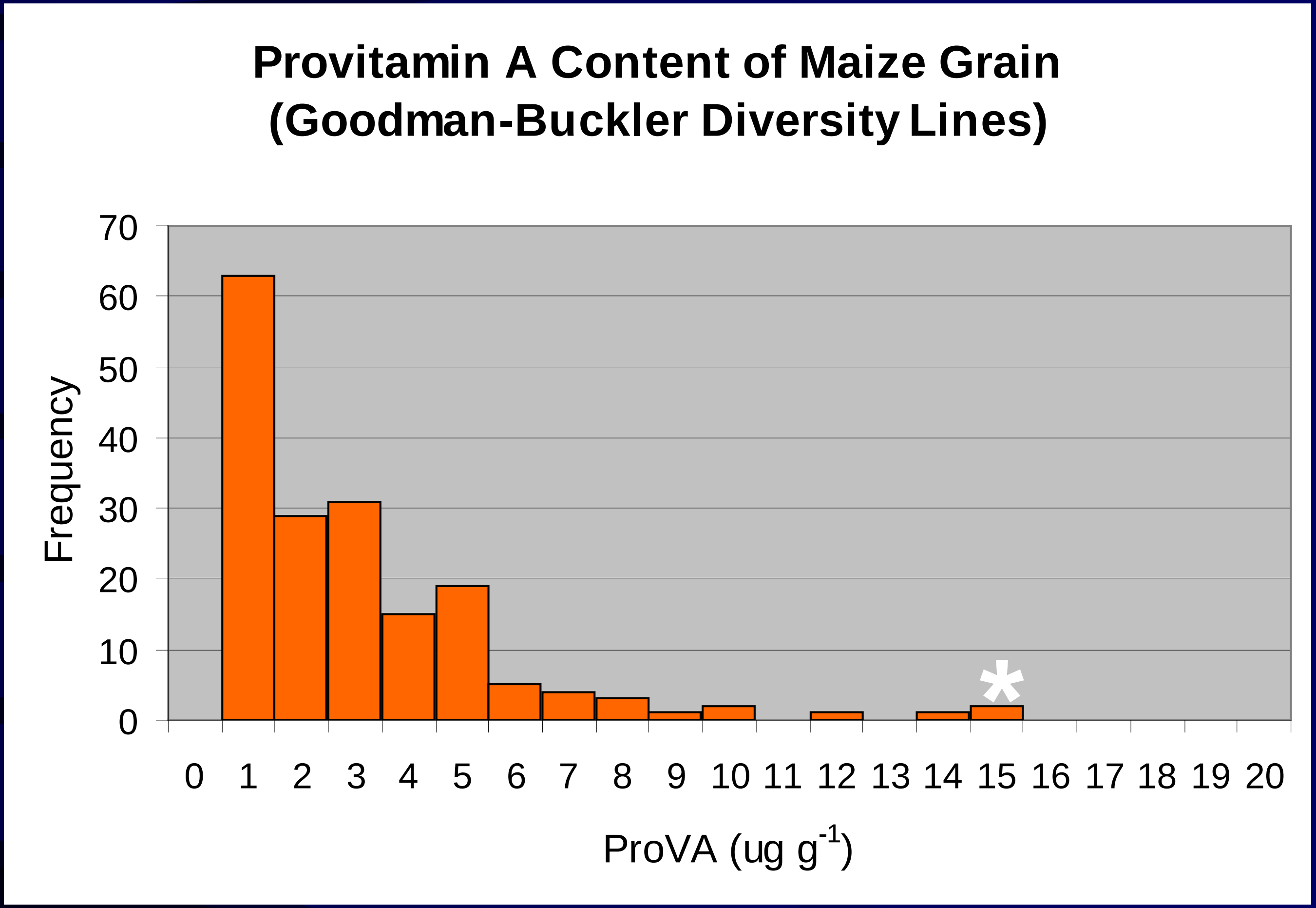
Yan/Kandianis……Rocheford Nature Genetics **42**, 322–327 (2010)

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)**



*!

22

Commercial hybrids

Orange Flints

ASMAS Breeding Lines S_2 generation

Advanced CIMMYT Coneventional Selection Breeding Lines

Cathy Kandianis

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

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-** [illegible]	[illegible]	[illegible]	[illegible]

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

Answer:
YES!

★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)

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

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 <u>***of just six-eight loci****</u> & 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
provitaminA 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

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
ROBIN STEVENS
DEBRA SKINNER
TYLER TIEDE
MEGAN FENTON

SULTANA ISLAM
CATHY KANDIANIS
JIANBING YAN
BRENDA OWENS

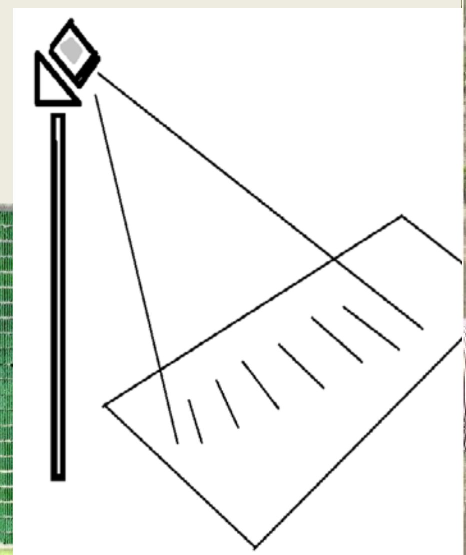
AND MANY, MANY OTHER GOOD SOULS….& INSTITUTIONS

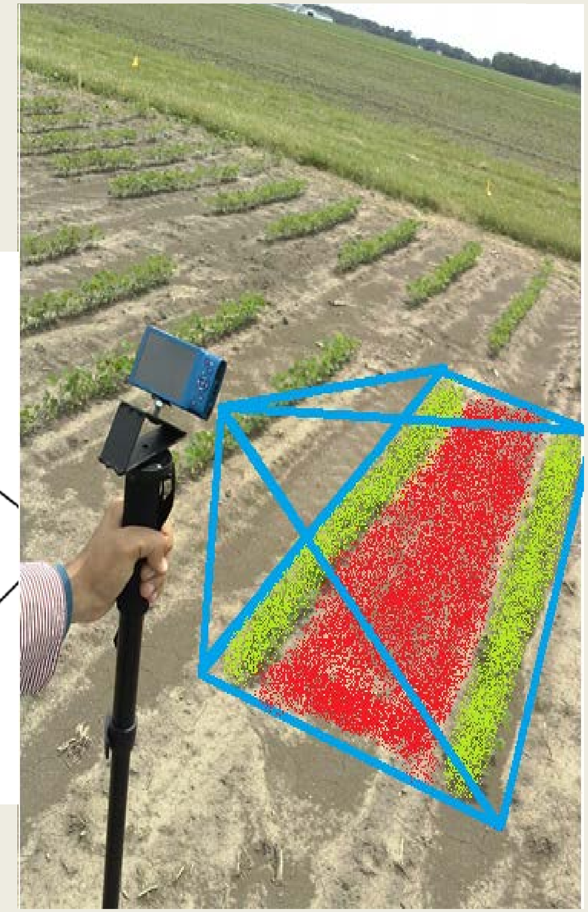
Unmanned Aerial Vehicles

- 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

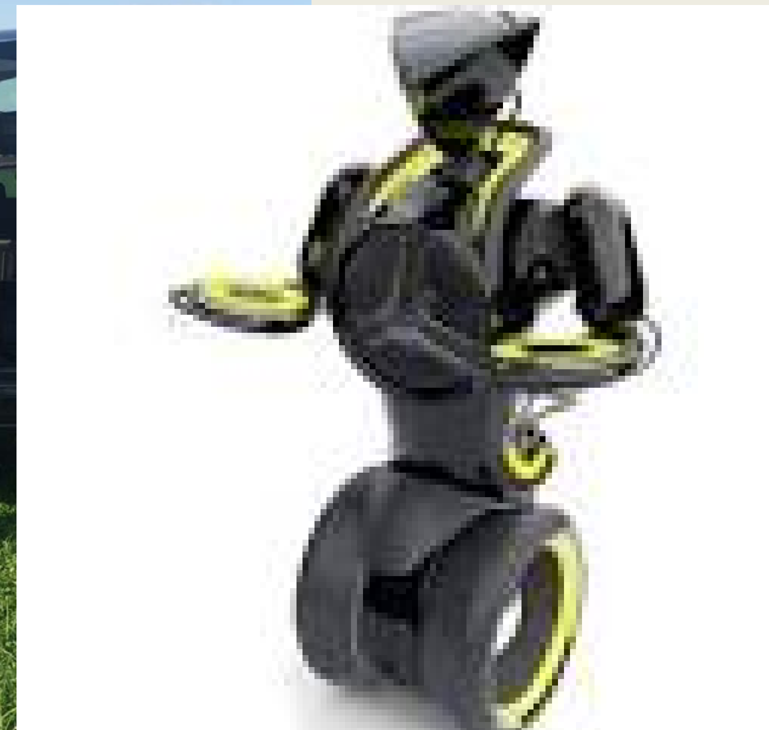
In future, maybe Robots will help Fly UAVs and Supercomputers
With Artificial Intelligence will Analyze and Interpret Results…

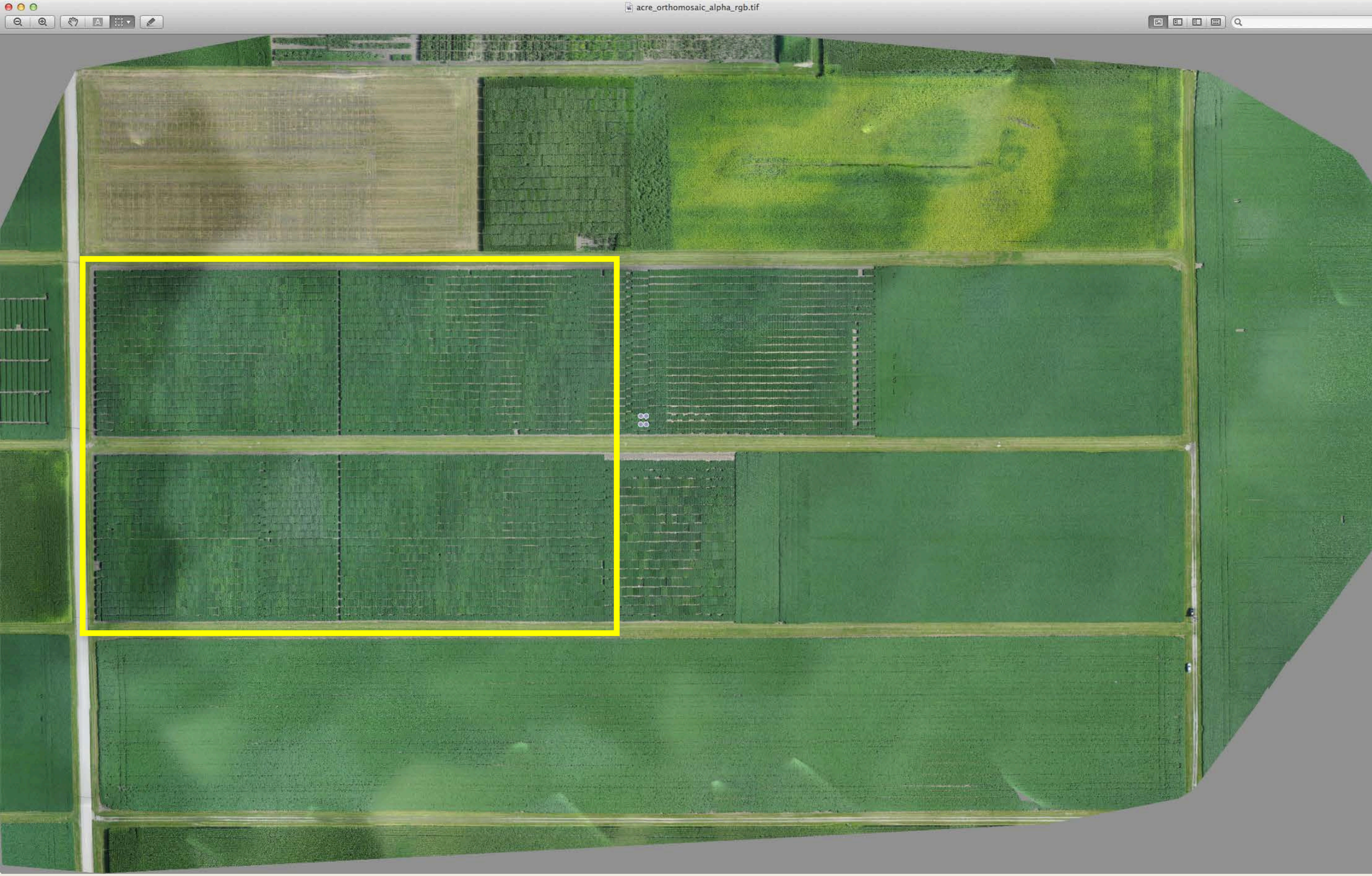

EXPERIMEN
AGX

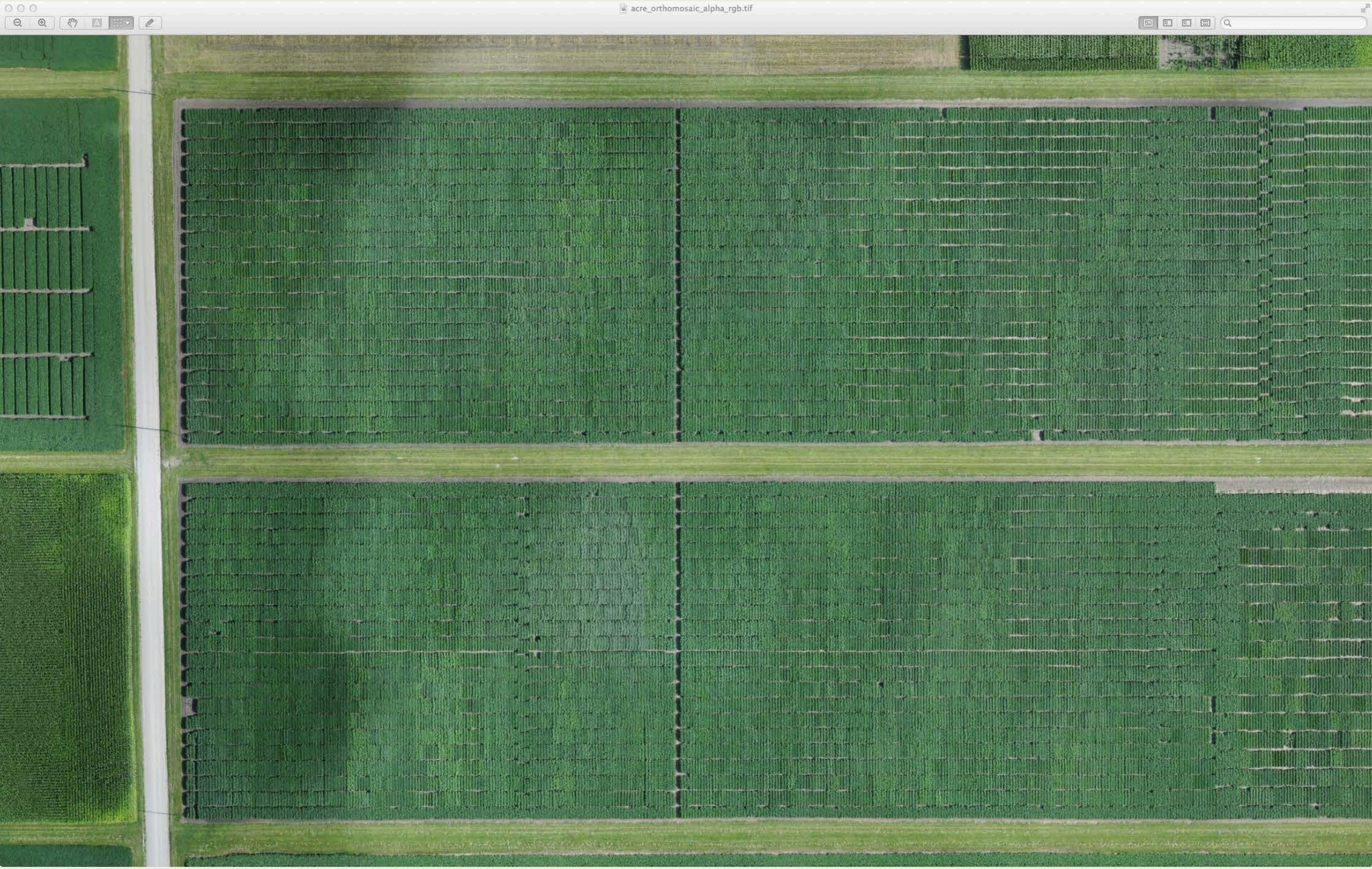

Grad Students and Postdocs have real intelligence, not artificial – very powerful, well usually……….

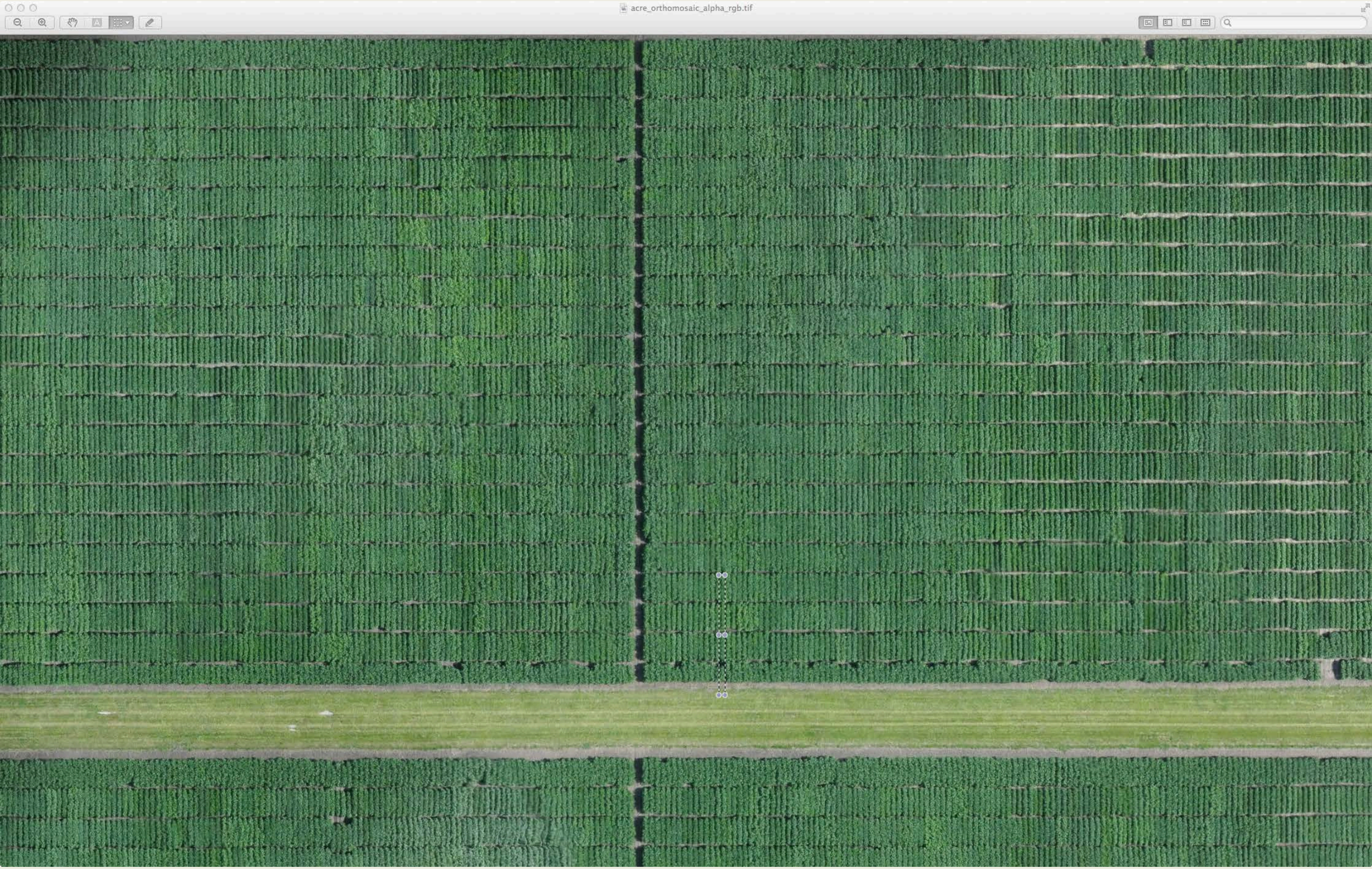
Dynamic Communicative Human Intelligence

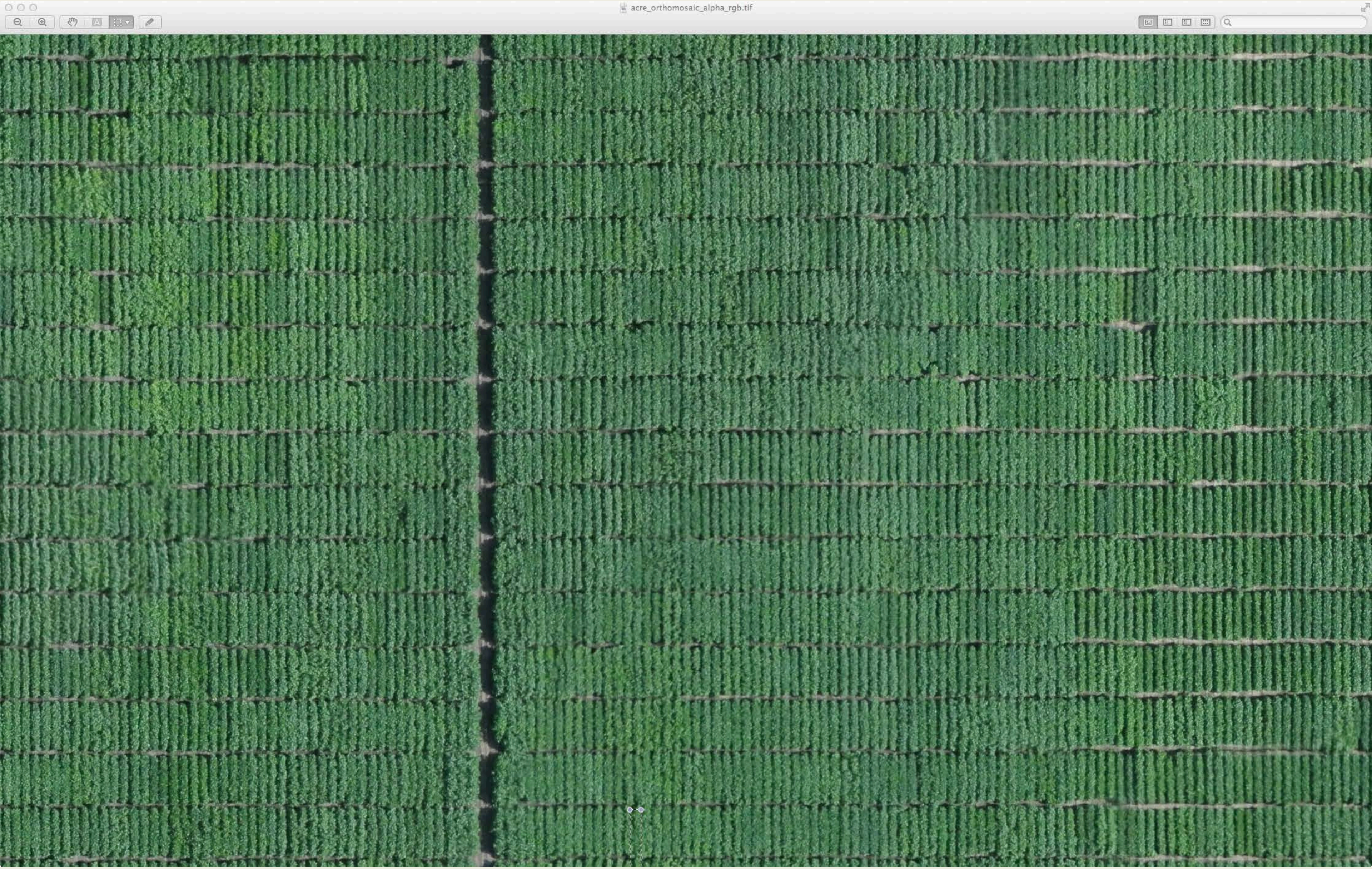


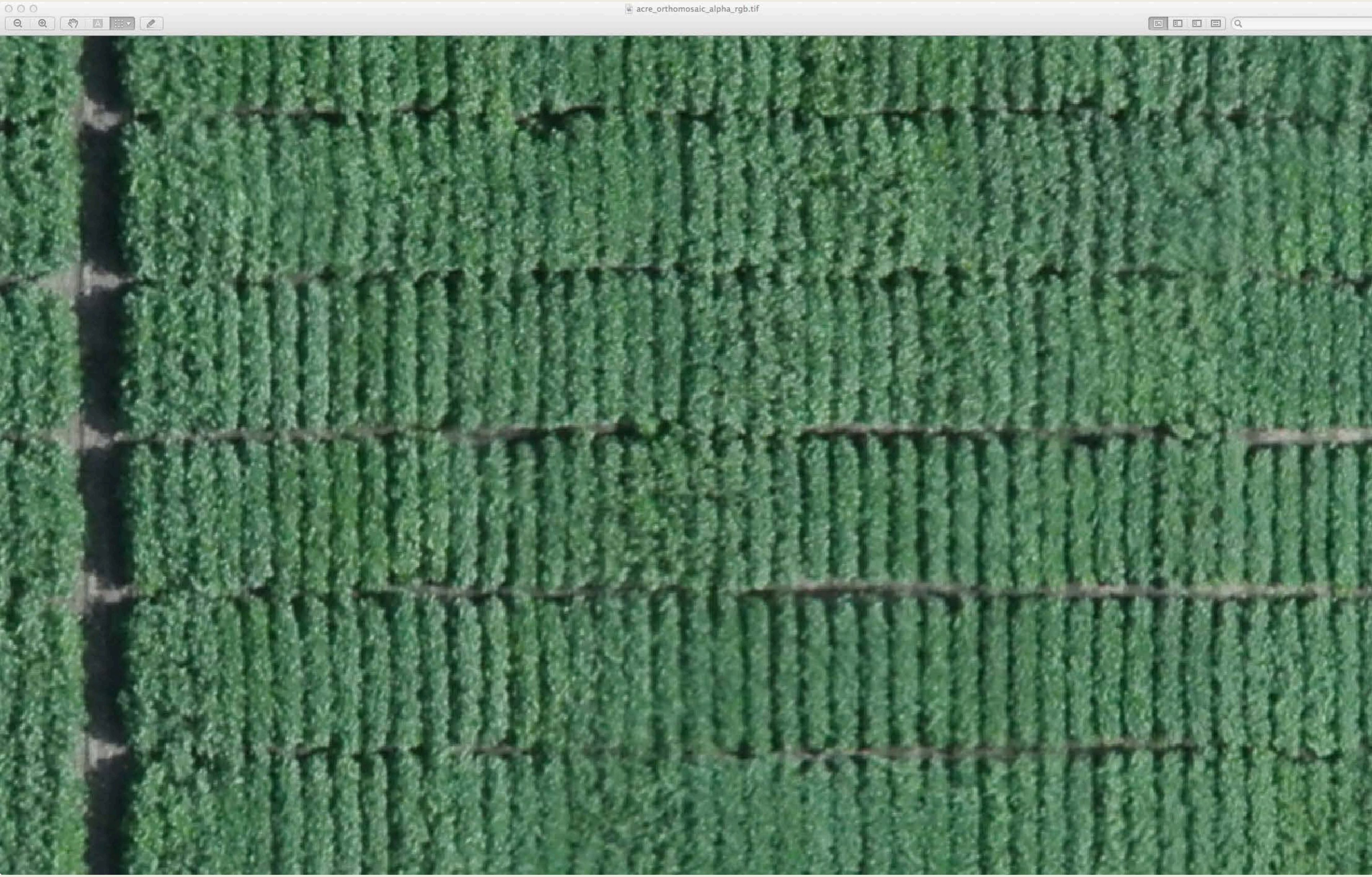


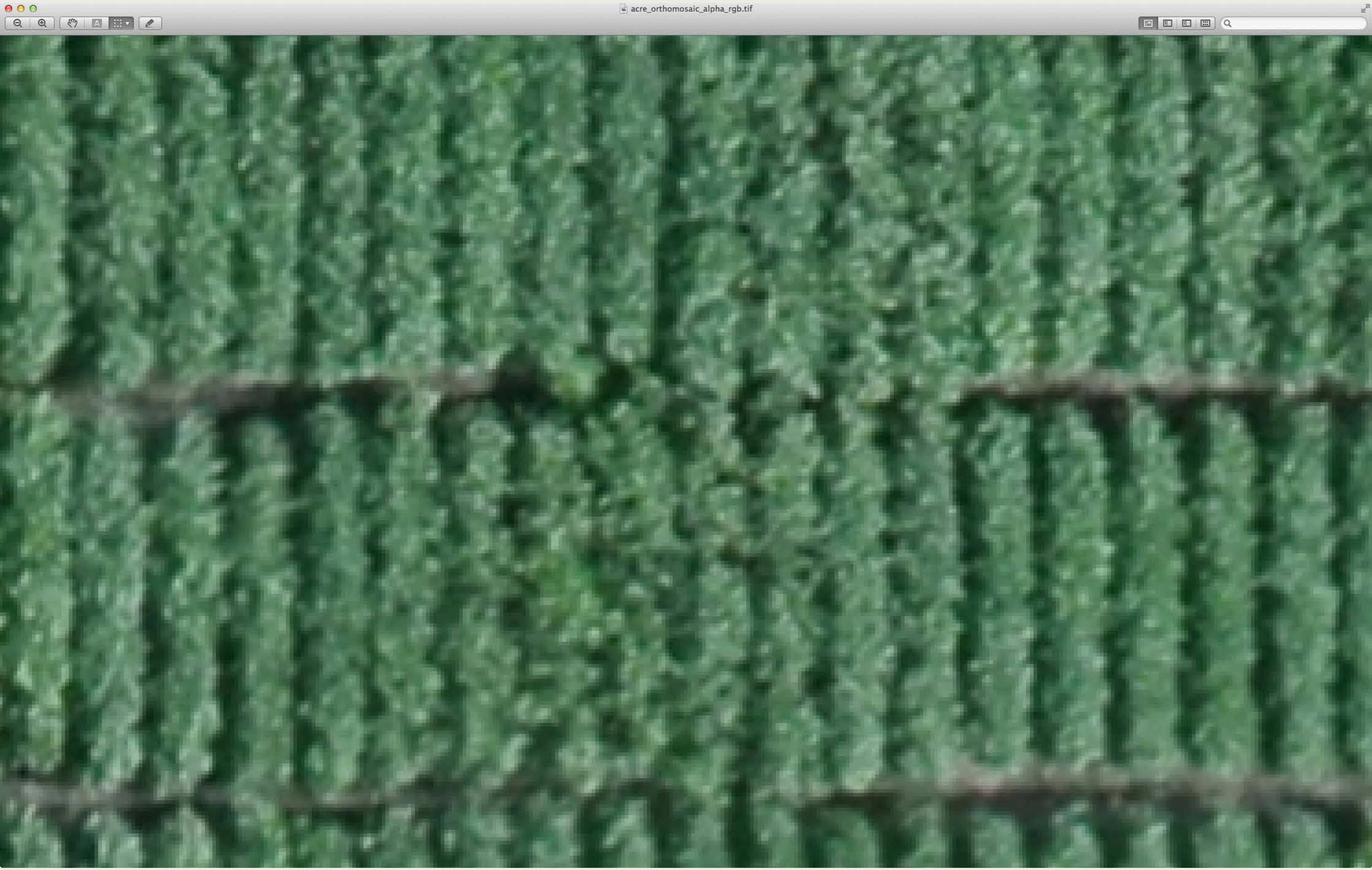

acre_orthomosaic_alpha_rgb.tif

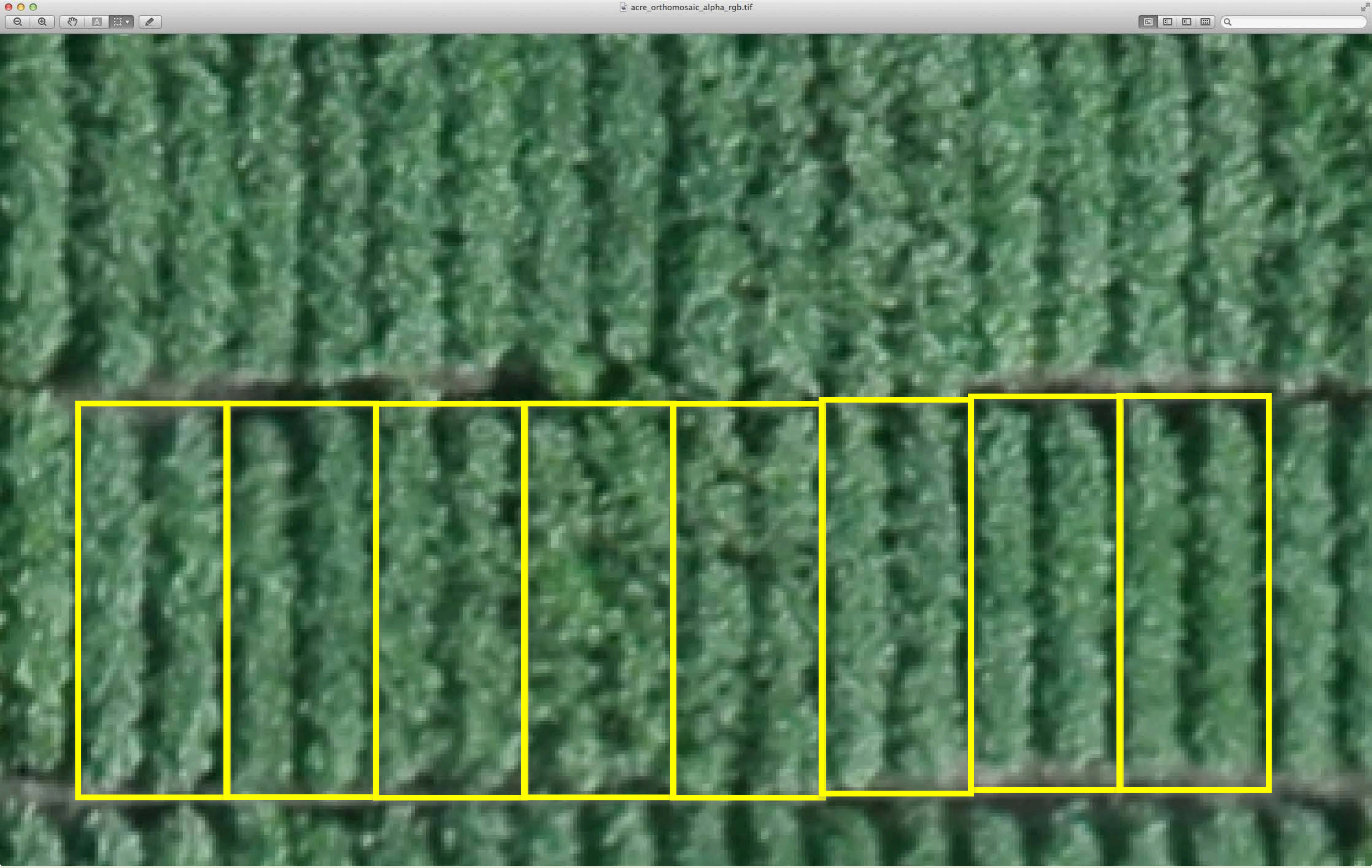

acre_orthomosaic_alpha_rgb.tif


acre_orthomosaic_alpha_rgb.tif


acre_orthomosaic_alpha_rgb.tif

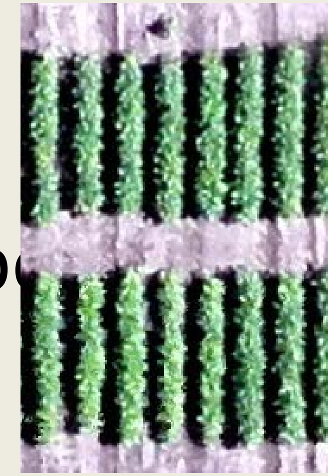

acre_orthomosaic_alpha_rgb.tif

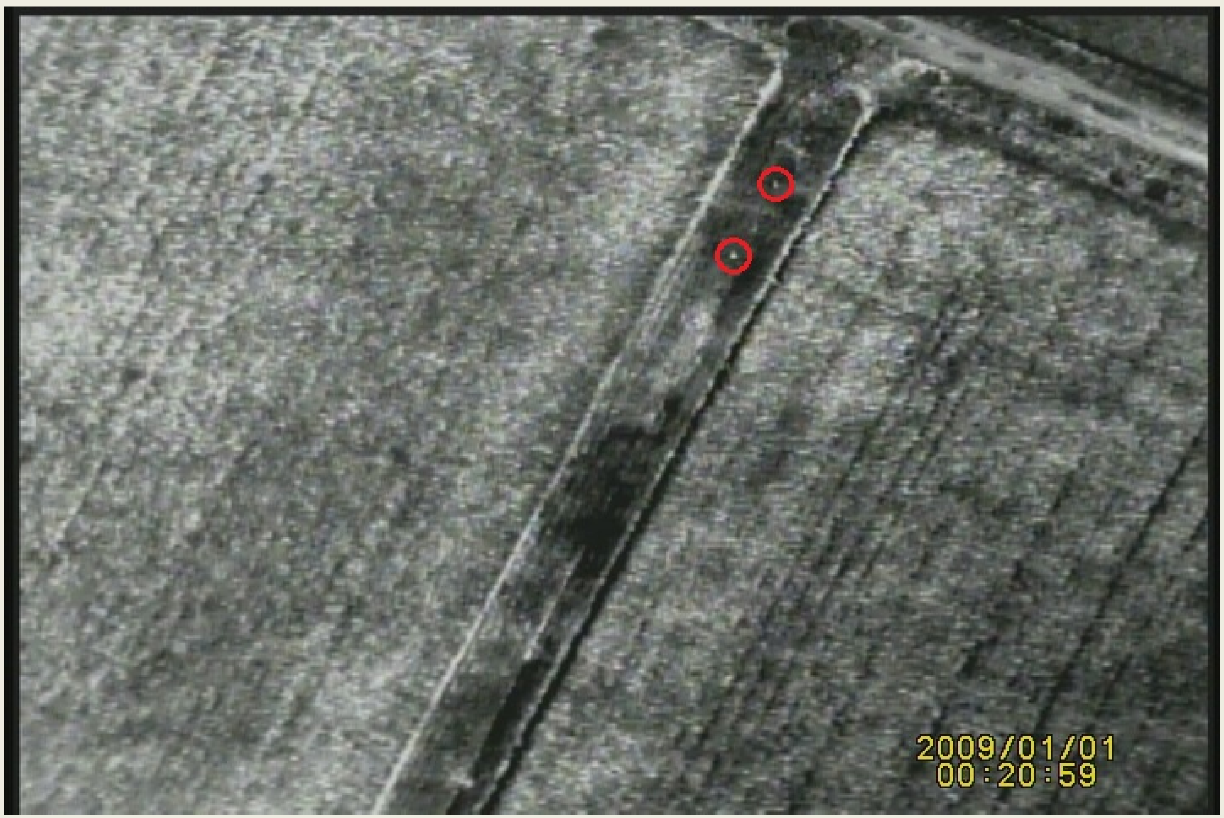

acre_orthomosaic_alpha_rgb.tif


acre_orthomosaic_alpha_rgb.tif

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!