OPEN TO THE PUBLIC

AGRICULTURE & DEVELOPMENT SEMINAR SERIES

5 JULY 2023 | 10:00 AM | SEARCA DRILON HALL, LOS BAÑOS, LAGUNA

BIOFORTIFICATION BREEDING FOR HIGHER PROVITAMIN A AND MACULAR CAROTENOIDS IN MAIZE FOR IMPROVED GLOBAL NUTRITION AND HEALTH

SPEAKER:

DR. TORBERT ROCHEFORD

Patterson Endowed Chair in Translational Genomics for Crop Improvement

Purdue University, USA

High ProVitamin A Carotenoids for Sub-Saharan Africa To Help Address Vitamin A Deficiency

Transitioned to

High Macular Carotenoids Lutein and Zeaxanthin for Ocular and Brain Health for USA

White

Almost No ProVitamin A: Beta-Carotene (BC)

or

Macular Carotenoids: Lutein (L) Zeaxanthin (Z)

Orange

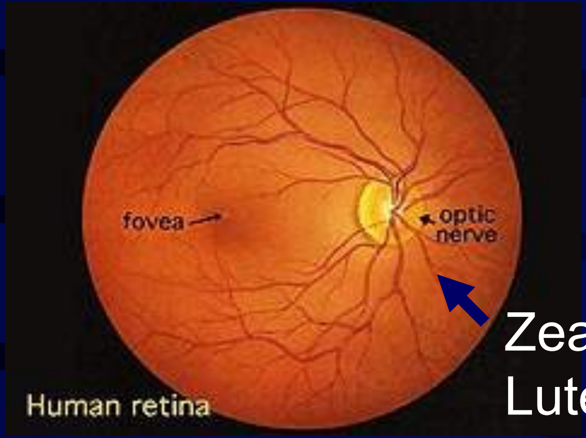
Lots of L/Z & BC Through Genetics,Breeding

Yellow

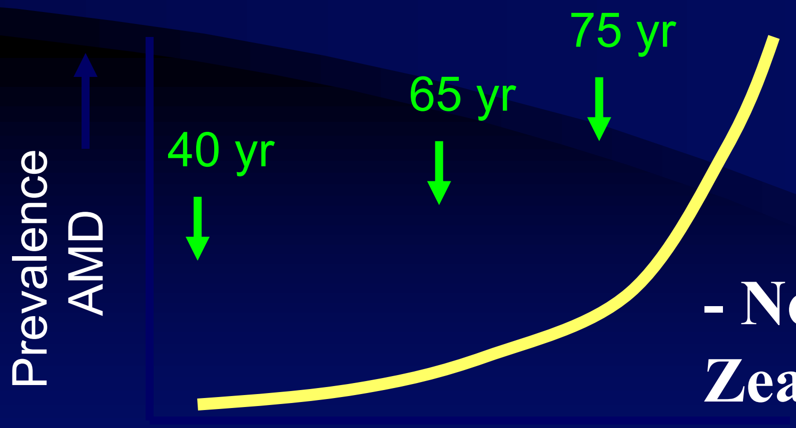
Very Little BC Some L/Z

Carotenoid Deficiencies are Globally Prevalent

US / Europe Age-related Macular Degeneration

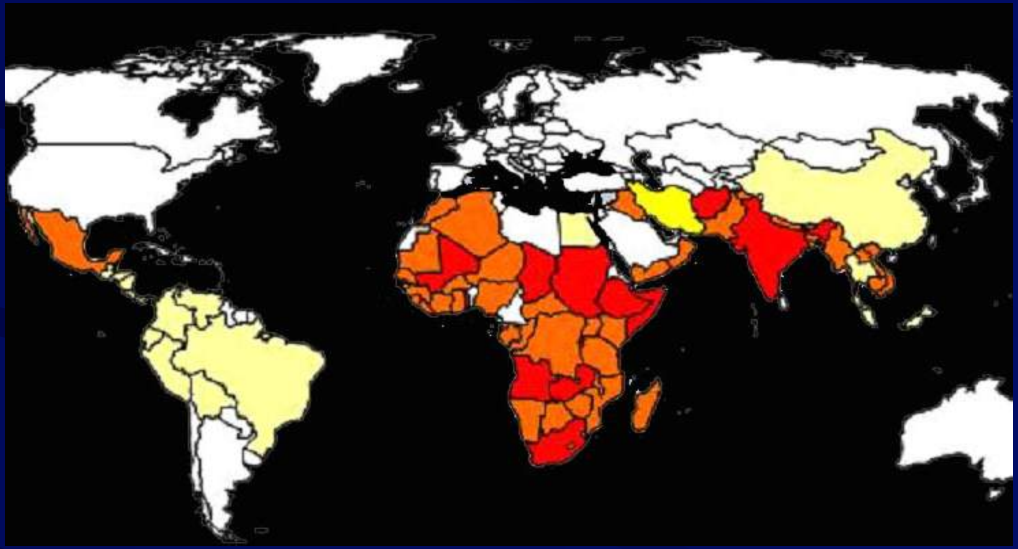


Zeaxanthin
Lutein



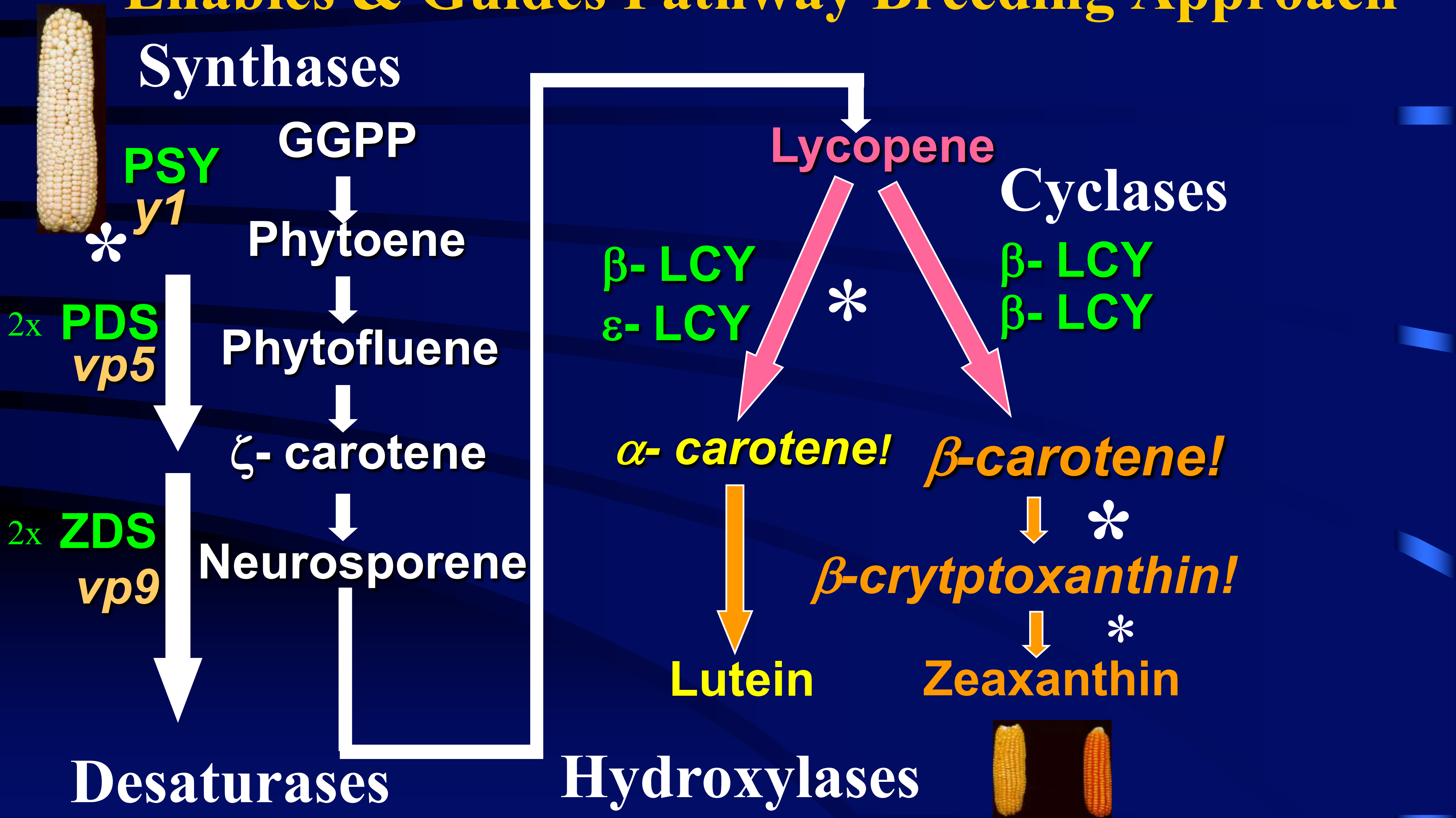
Many Countries: Vitamin A Deficiency

Beta-Carotene
Alpha-Carotene
Beta-Cryptoxanthin



- Now Well Documented Benefits of Lutein & Zeaxanthin on Visual & Brain Health, *and* Cognition

Carotenoid Biosynthetic Pathway – Enables & Guides Pathway Breeding Approach



Using Natural Diversity

Before My Keynote Talk at 25th Anniversary European EUCARPIA Corn & Sorghum Breeding Meeting in Serbia, 2022 –

I Surveyed Current Use in Sub-Saharan Africa of Favorable Alleles of Carotenoid Genes I was involved in Discovery of, for:

Marker Assisted Selection for Higher ProVitamin A

Progress has been <u>*Outstanding*</u>, therefore presented their work:

As a Nice success story of genetics to genomics to breeding to field to applications to food to people.....

Presenting Followup Efforts from:

Thoko Ndhlela

Center for Maize and Wheat Research (CIMMYT) Zimbabwe

Thoko Ndhlela, Manje Gowde using *crtRB1*

Abebe Menkir

Melaku Gedil

Institute for Tropical Agriculture (IITA) Nigeria

Abebe Menkir, Melaku Gedil using *LycE* and *crtRB1*

Selection for *weak* alleles, less mRNA transcription thus less enzyme, of:

crtRB1 – beta-carotene hydroxylase

LycE – lycopene epsilon cyclase

Starting Point - Survey of 100 Africa CIMMYT Zimbabwe Maize Breeding Lines for Carotenoids in 2002 (Pixley et al.)

	Average ug/g	Maximum ug/g
Beta-carotene *trans*	***1***	***6***
Beta-carotene *cis*	***1***	***7***
Beta-cryptoxanthin	**1**	**6**
Alpha-carotene	**0.2**	**2**
(these three 0.5x)		

ProVitamin A	**2.6**	**8.8**
Non-ProVitamin A Carotenoids		
Lutein	**7**	**32**
Zeaxanthin	**9**	**35**

Selection of weak LycE allele sends flux to Beta-Branch of pathway, and thus low Lutein

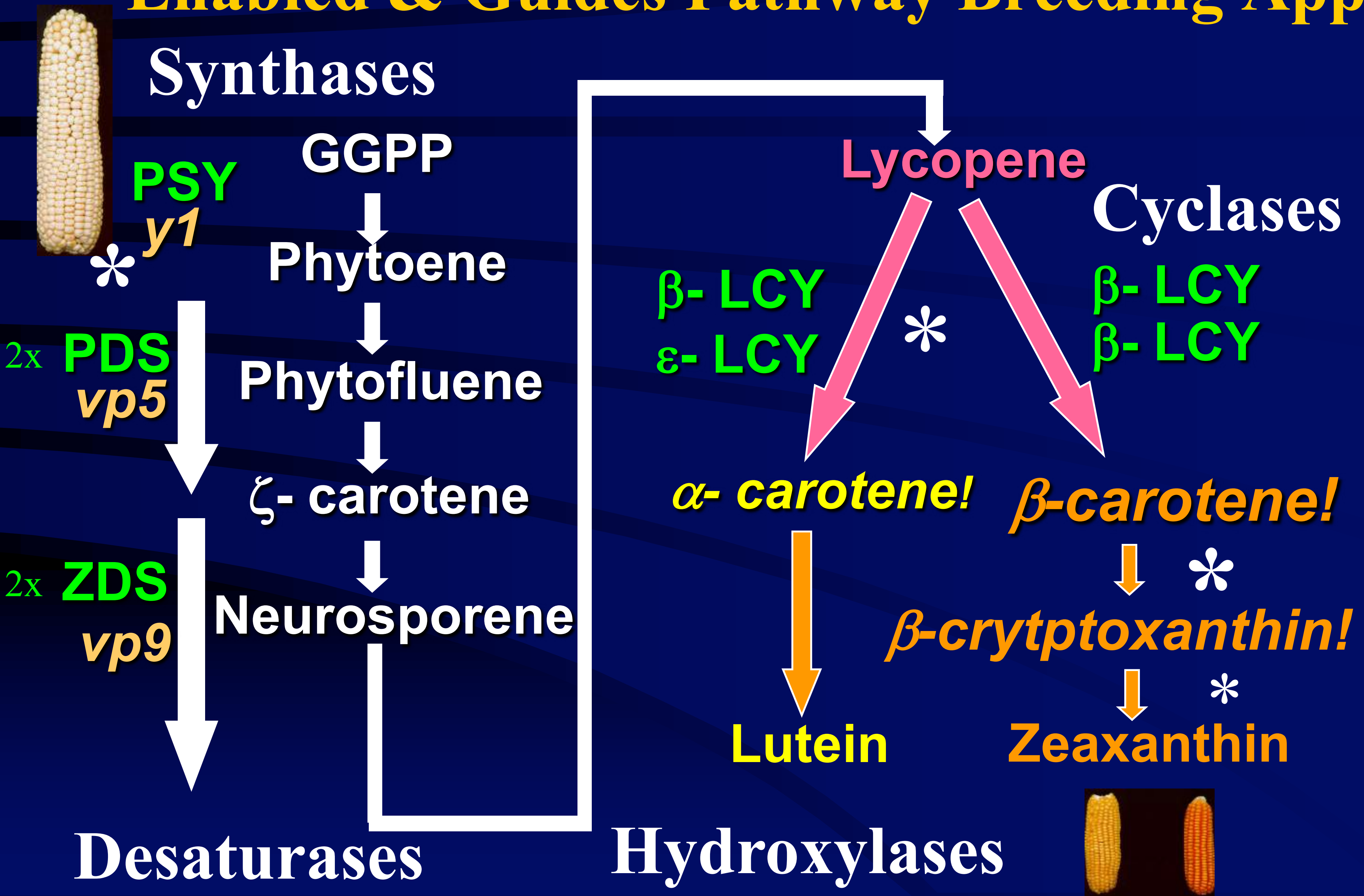
Selection of crtrb1 slows flux at Beta-Carotene and thus less Zeaxanthin

Combined Result is *High* Beta-Carotene, ProVitamin A and *Low* Lutein and Zeaxanthin

This was ***either/or*** *option through allele selection*

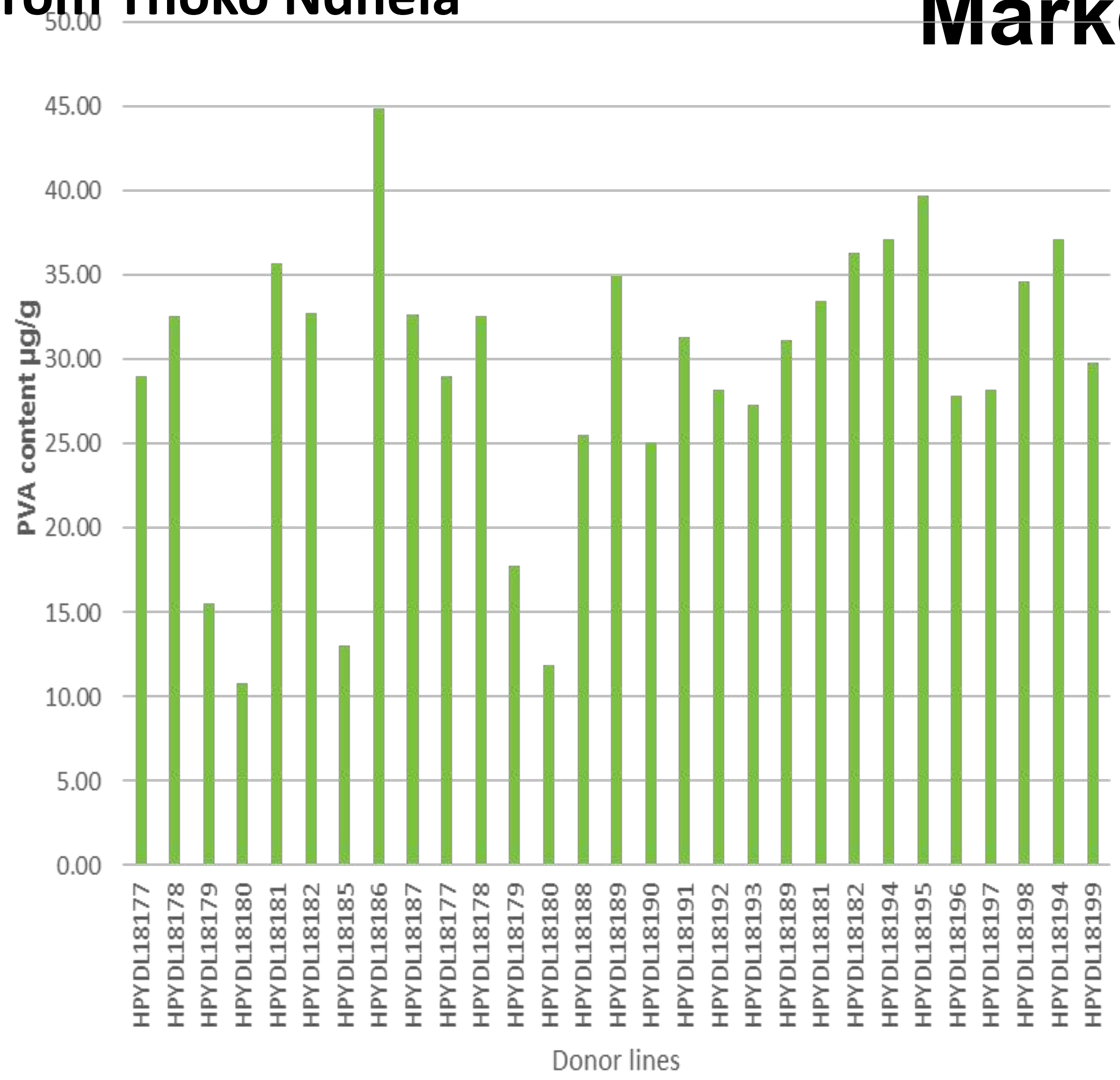
Now, they are fixing the crtrb1 allele early generation and selecting visually for orange (and indirectly total carotenoids) and selecting with HPLC for proVitamin A

Carotenoid Biosynthetic Pathway – Enabled & Guides Pathway Breeding Approach



CIMMYT, Harare, Zimbabwe, From Thoko Ndhela

Donor Lines Generated with Marker Assisted Selection



PVA content in selected donor lines

- **Donor lines developed partially from temperate germplasm gives yield, crtrb1**
- **Highest ≥ 44 µg/g PVA content**
- **Many over 30 ug/g – Double H+ Target**

Plant No.	Pedigree	S10_134583972	S10_134655704	S10_136072513	S10_136840485	S10_137904716	SYN11355	PVA_Selection
1	(CL107253	G:G	C:C	T:T	C:C	C:C	A:A	Select
2	(CL107253	G:G	C:C	T:T	C:C	C:C	A:A	Select
3	(CL107253	G:G	C:C	T:T	C:C	C:C	A:A	Select
4	(CL107253	G:G	C:C	T:T	C:C	C:C	A:A	Select
5	(CL107253	G:G	C:C	T:T	C:C	C:C	A:A	Select

White Elite Line Conversions

White x Orange Donor

White is Elite In Zimbabwe

1[st] Backcross To Orange

BC1F1

Remember Orange Donor fixed CrtrB1

2[nd] Backcross to Orange Donor

BC2F2- Marker Assisted Selection for CrtB1

Crtrb1 SNP Genotyping

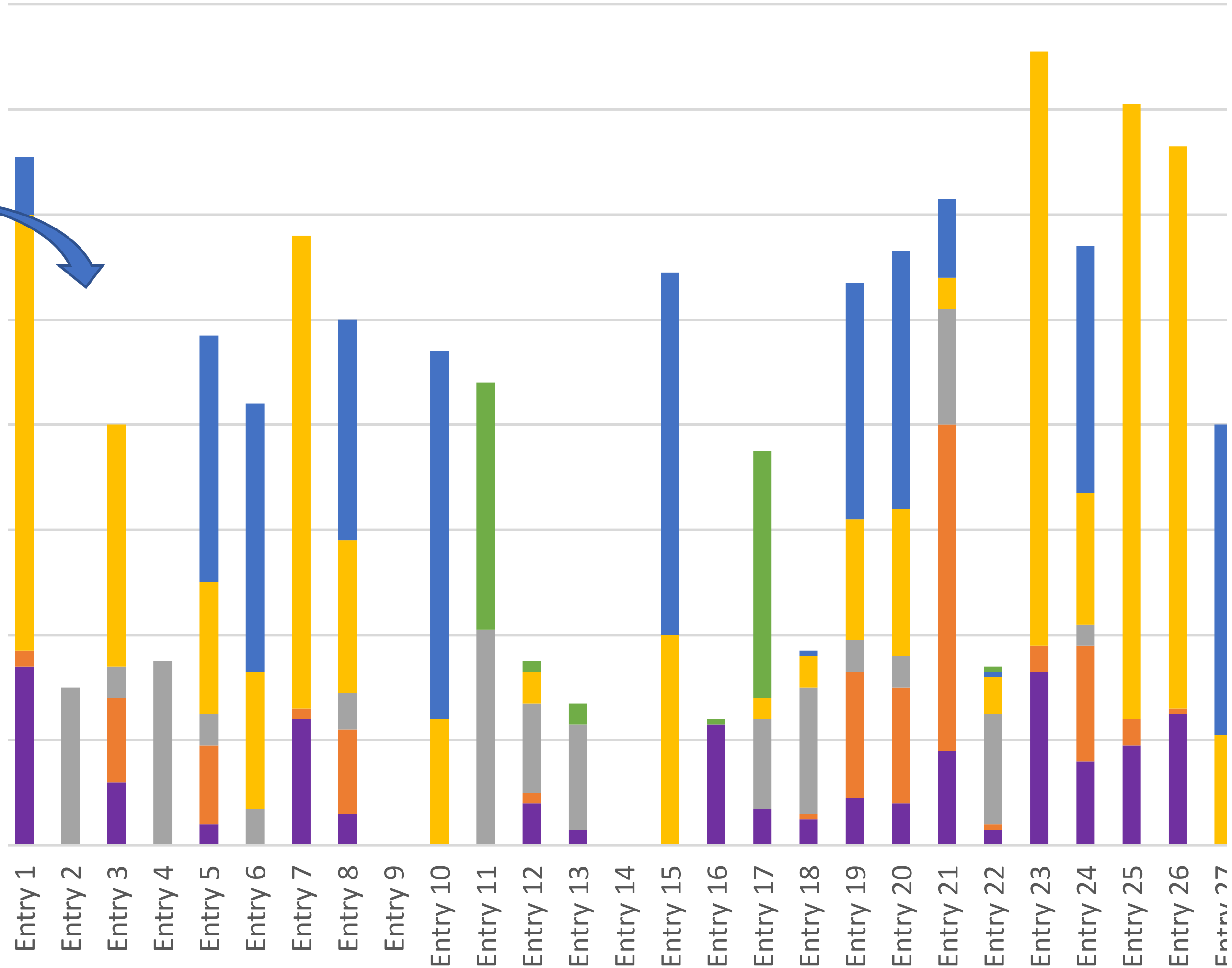
BC2 to homozygous crtb1 donor

Plant No.	Pedigree	S10_134583972	S10_134655704	S10_136072513	S10_136840485	S10_137904716	SYN11355	PVA_Selection
1	(CL107253	G:G	C:C	T:T	C:C	C:C	A:A	Select
2	(CL107253	G:G	C:C	T:T	C:C	C:C	A:A	Select
3	(CL107253	G:G	C:C	T:T	C:C	C:C	A:A	Select
4	(CL107253	G:G	C:C	T:T	C:C	C:C	A:A	Select
5	(CL107253	G:G	C:C	T:T	C:C	C:C	A:A	Select

Backcrossing to the orange donor provides high total carotenoids.

Large populations used - allows selection for other agronomic traits.

Seedling genotyping

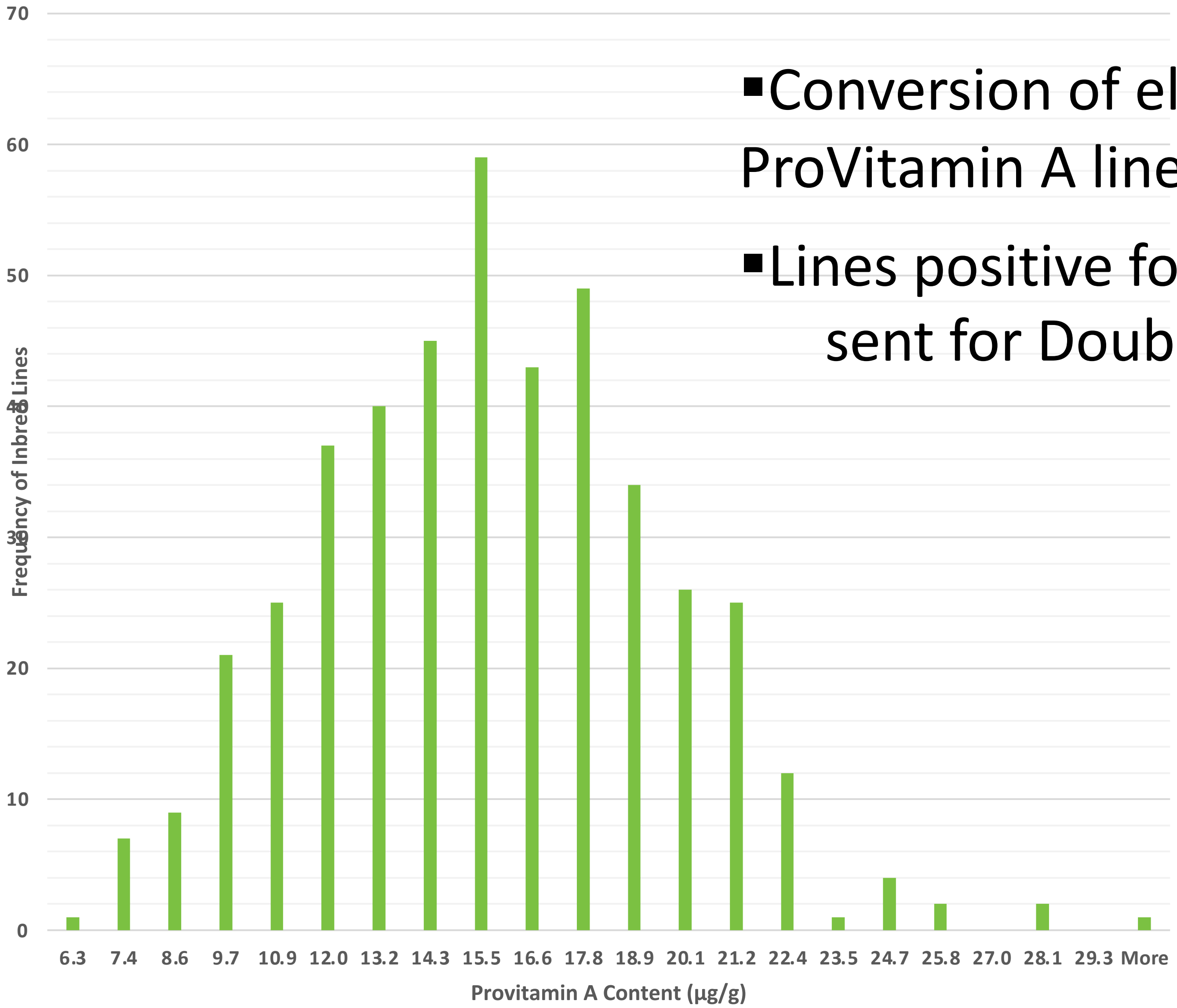


- **3-4 leaf discs collected per plant**
- **Genotyping results in time for the breeder to perform the selections in the field**
- **Populations homozygous for CrtRB1 & MSV1 (virus resistance) alleles sent for DH induction**

Entry - means a population derived from a cross

Mike Olsen et al. CIMMYT Excellence in Breeding

Genetic gains in inbred line development



- Conversion of elite non-high ProVitamin A lines to high ProVitamin A lines with MAS
- Lines positive for Homozygous crtRB1 Favorable Allele sent for Double Haploid (DH) Inbred Induction

Genetic variation of provitamin A concentration developed in more agronomically elite inbred lines

New Doubled Haploid (DH) Lines

Abebe Menkir et al., IITA, Nigeria, in 2020 surveyed Inbreds from Breeding program after Years of Selection for High ProVitaminA

Used Multiple Approaches –

-Visual Selection for Dark Orange Kernel Color

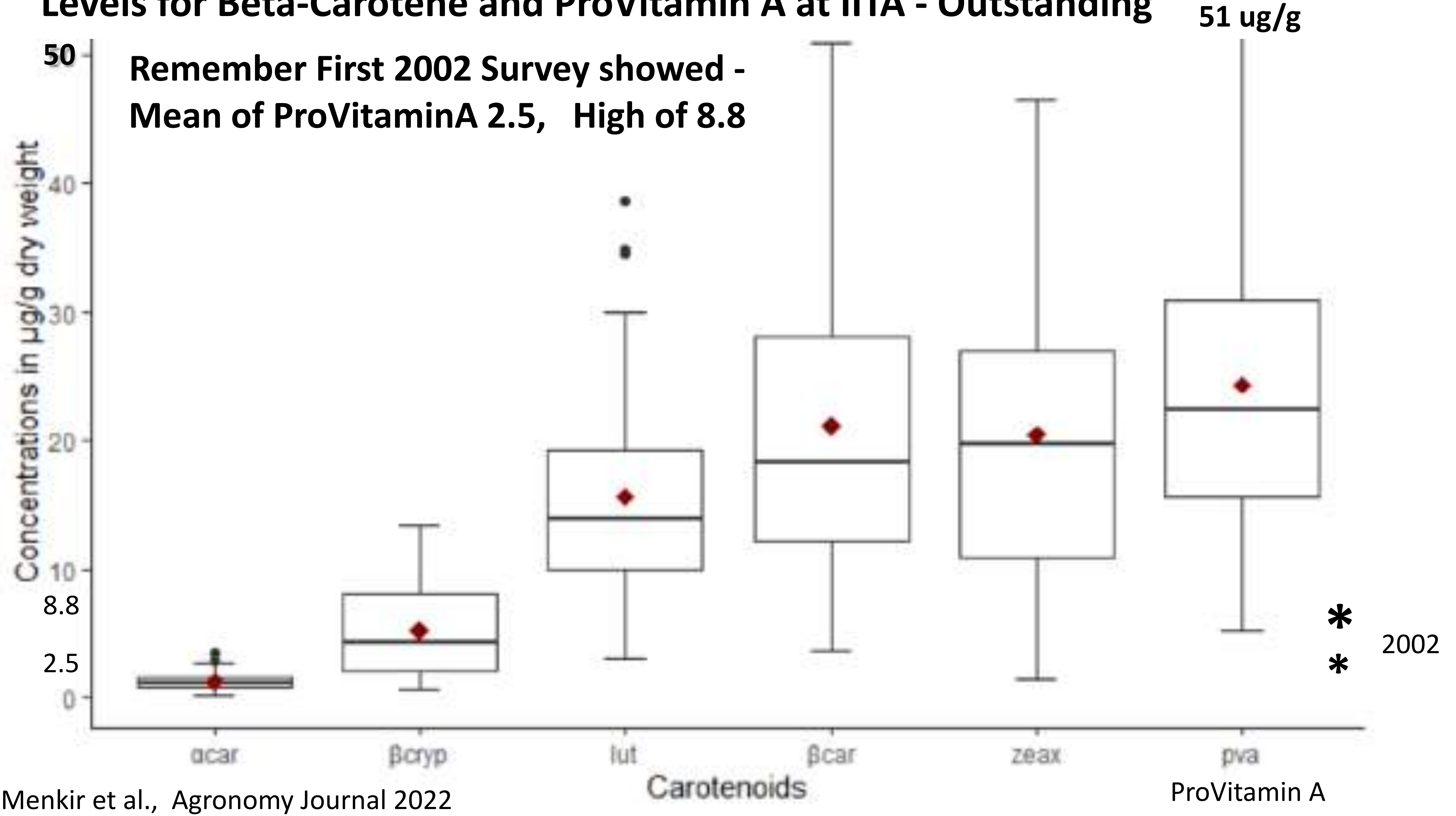
-High Performance Liquid Chromatography (HPLC) based selection for higher ProVitaminA

-Marker Assisted Selection with Favorable LycE and crtRB1 Alleles

They Have Made Outstanding Progress for High Levels of ProVA

Importantly, they also produced lines high in BOTH beta-carotene AND lutein and zeaxanthin. ***This is new, notable, important*.**

Levels for Beta-Carotene and ProVitamin A at IITA - Outstanding



Menkir et al., Agronomy Journal 2022

Created First Time High Beta-Carotene AND Also High Lutein, Zeaxanthin

Preventing Blindness & Death is the Priority of course. Yet then - Providing Macular Carotenoids for Visual & Brain Health, Cognitive Function is Logical

Inbred	Carotenoids (μg/g Dry Weight)						Genotype *					
							crtRB1			LCYE		
	Lutein	Zeax.	βcry	αcar	βcar	pva	3′TE	InDel4	5′TE	3′indel	5′TE	SNP (216)
IITATZI1653	3.0	1.4	0.9	0.6	51.0	51.7	1/3	0	2/1	0	2	2
IITATZI1715	6.8	4.6	0.7	0.4	45.3	45.8	1	0	2	8	2	2
IITATZI2071	26.0	10.2	1.3	0.7	42.4	43.3	1	0	2	8	2	2
IITATZI2066-2	16.3	10.6	1.9	0.8	42.0	43.4	1	0	2	0	2	2

Production of 27,337 tons of certified seeds of provitamin A biofortified maize varieties in Ghana, Mali and Nigeria from 2014 to 2021

Seed planted ~ 1,118,332 hectares, benefited 2,062,236 households

Nigeria national food consumption and nutrition survey in 2021 and 2022 found 13.4% of respondents regularly consumed provitamin A biofortified orange maize in a variety of foods

Consumption provitamin A biofortified maize was 38% in the Northeast of Nigeria due to support programs from Nigerian government and USAID

Seed companies are benefiting from sales of biofortified maize seed

This is in Zambia, yet in my view – The Entire World ‘Should’ Look Like This

Transition to Breeding Orange Corn for America

Zambia about early 2015, some from HarvestPlus (H+) were talking with a government official in Zambia -

He asked: “So you want us to Grow and Eat this Orange Corn, do you Grow and Eat Orange Corn in United States?”

The answer was “No” unfortunately.

Caused government official to Wonder something to the effect…..“Why do you not eat this in USA, is something wrong with Orange Corn?”

To Develop Orange Corn for USA and Reach Consumers –

Requires Entrepreneurial Start-Up Company Approach

Large Multinational Seed Corporations (*which influence a significant portion of the global food supply*) are not going to touch a new unique nutritional hybrid, which requires identify preservation, unless…..

…there is about $5 million dollars in sales in hand or close to in hand. Otherwise they will *not* pursue.

NutraMaize

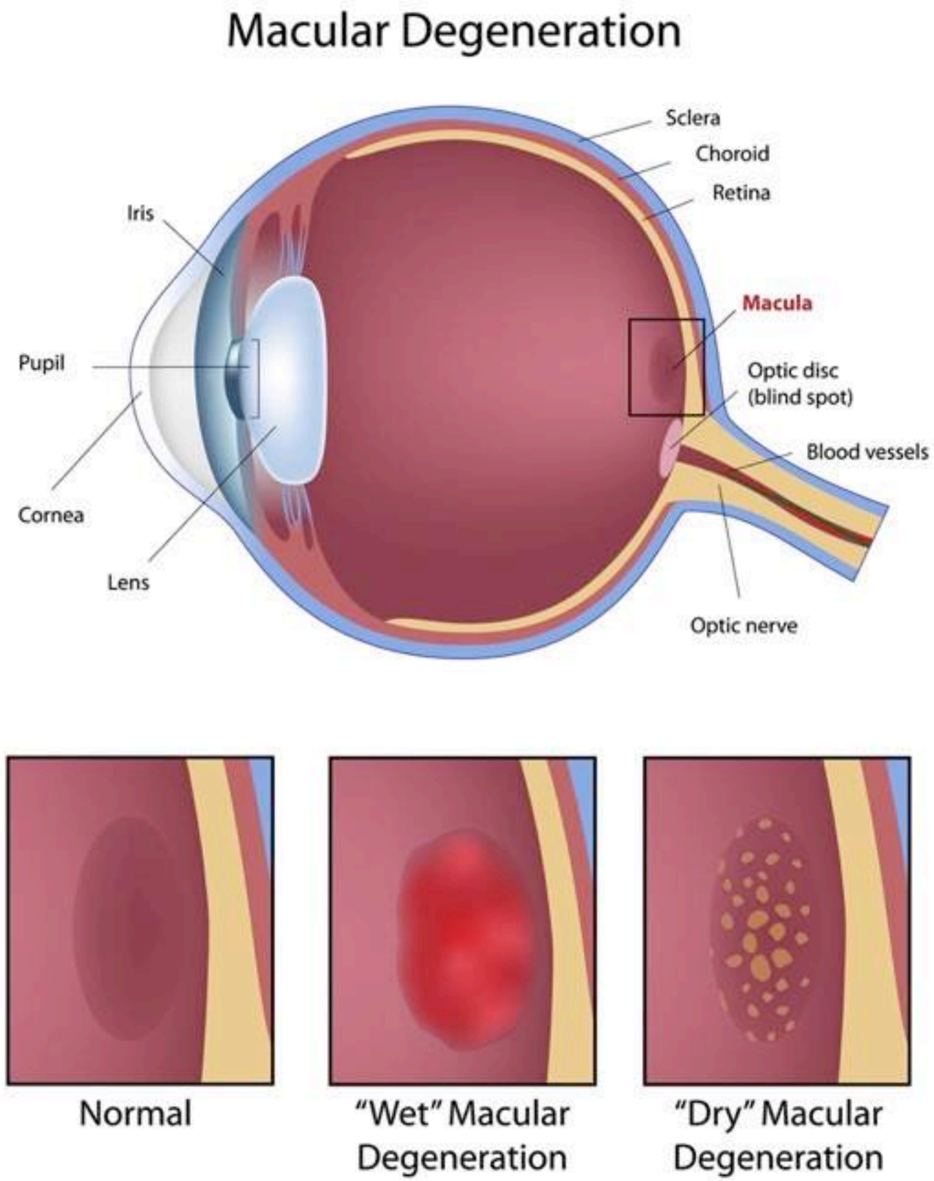
IMPROVING HEALTH THROUGH AGRICULTURE

A Purdue Technology Affiliated Startup

Enabled through Purdue Research Foundation Resources:
LaunchBox now FireStarter
Foundry Grounds
Spirited Entrepreneur
Office of Technology Commercialization

External Funding up to this point from HarvestPlus & NSF Plant Genome Research Program

Moving Forward from this point, Funding largely NSF STTR Phase I & II, USDA NIFA Phase I (&II)



Vitamin A Deficiency is Not a problem in USA

But Age Related Macular Degeneration (AMD) *Is a PROBLEM*

5.5+ million people in USA affected

AMD: 2 million **Early stage: 3.5 million**

Xanthophylls (*which are present in orange corn*) have been found to effectively reduce risk of AMD

Formed NutraMaize, LLC with Evan Rocheford, CoFounder & CEO

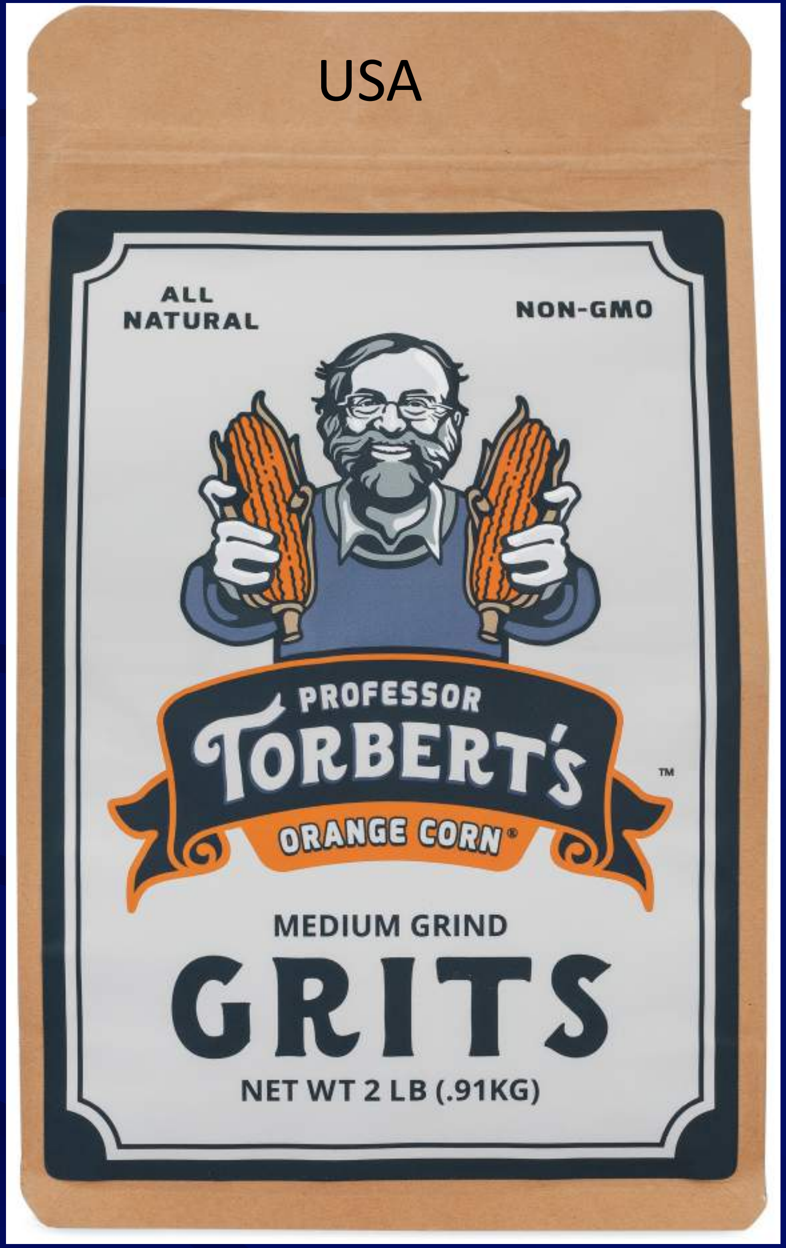
Initial product line: Professor Torbert's Orange Corn

Marketing is Important –

Orville Redenbacher's Popcorn

Burt's Bees products


Zimbabwe
NEW
Sky Brands
1KG
Orange Breakfast Meal
ORIGINAL
Naturally Rich in Vitamin A
Sky Brands
Factory 101, Chitungwiza, Harare
0775253231 / 0775078490
skybrands2018@gmail.com
PROUDLY ZIMBABWEAN
ORANGE MAIZE PORRIDGE
Eat Healthy . Stay Healthy


USA
ALL NATURAL
NON-GMO
PROFESSOR
TORBERT'S
ORANGE CORN
MEDIUM GRIND
GRITS
NET WT 2 LB (.91KG)

Chef Mimi J Texas
Instagram
Influencer

Developed Milled Products
from Flinty Orange Corn

Grits, Cornmeal, Corn Flour

Differ by particle size, coarseness

Grits largest particles
Cornmeal intermediate
Flour finest particles

Polenta is intermediate to Grits & Cornmeal

Polenta – Italy – Northeast USA

Grits – Southern – Starting with Colonists introduced to ‘Mush’ by Indigenous People

Orange Grits have ”creamy texture” , “nutty” “buttery” flavor (*online Amazon reviews*)

Sensory perceptions may be related to:

- flintiness / vitreousness
- high levels of carotenoids

- Chefs, Restaurants, Food Service
- Online Amazon
- Retail
- Covid Impacted
- Future: School Lunches, Hospitals

White

Dent

Orange

Is Flinty

Yellow

Dent

Tell Children “***Orange Corn Tastes Good***”, and they try it and like it. Only then do you tell them “***it is good for you***”

Tell Children “***Orange Corn is Good for You***”, they won’t eat it. Children associate good nutrition with things like kale, spinach, vegetables……that most children don’t like

Recipes - www.professortorberts.com
GoDaddy.com did website for free, learn more after seminar from Evan Rocheford at Table in Foyer. Along with delicious Lemon Orange Polenta Cookies made by Katie Rocheford…

Click on Recipes on the first page. When you scroll down pictures, if you want recipe, then click on the picture, and the written recipe comes up.

Economic Considerations

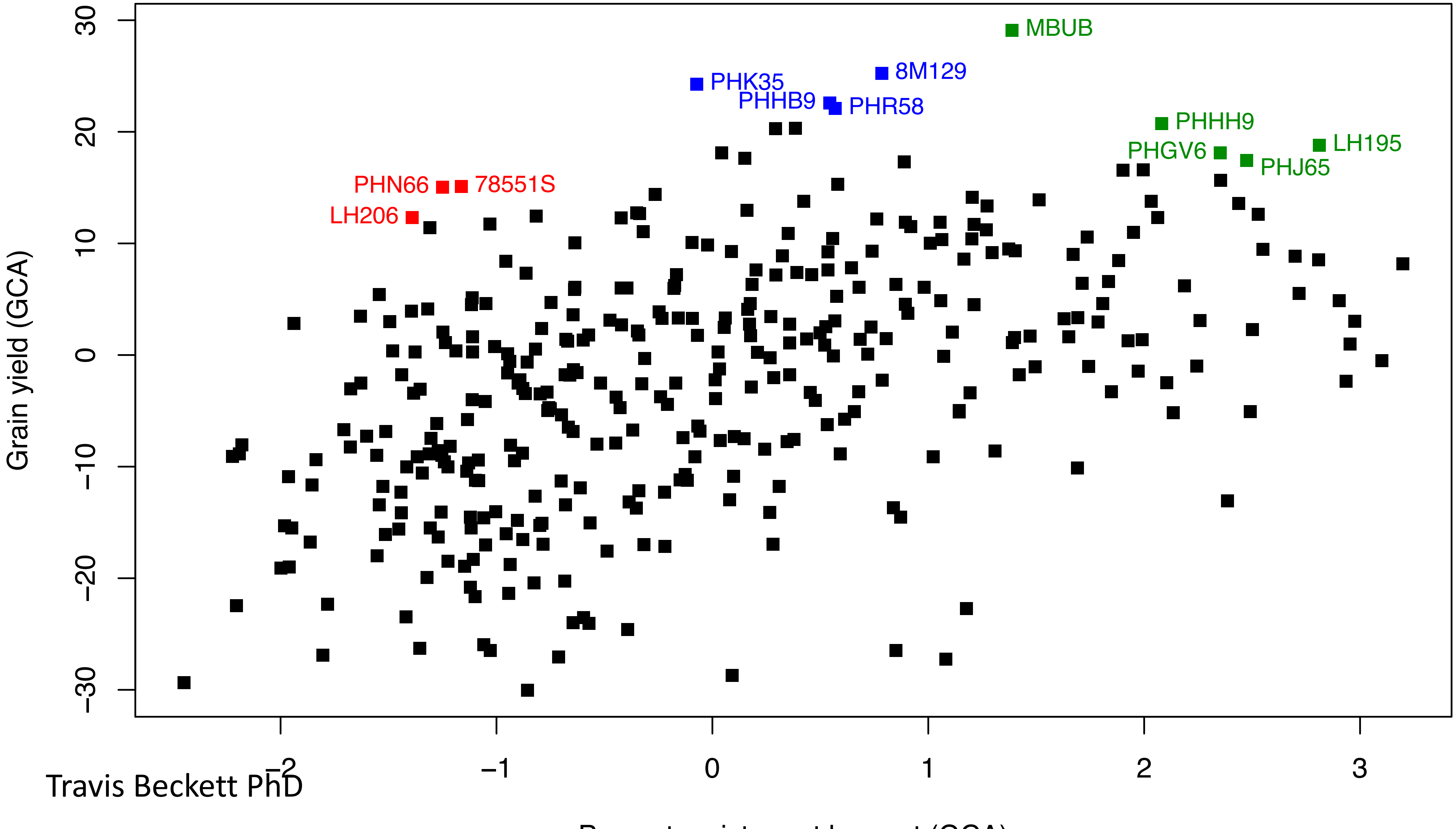
- For Orange Corn to be economically viable need to have certain grain yield levels

- Started breeding for USA with germplasm adapted to Asia and Africa. So had some work to do to breed for adaptation and good grain yields

- This has been, and will continue to be a major priority

- Took advantage of previous research and development on former commercial Plant Variety Protected inbreds (exPVP)

Used Valuable Data from Yield Trials of former Plant Variety Protected (ExPVP) Commercial Inbreds



Picked some mid-maturity inbreds that performed well to work with

Travis Beckett PhD

Yield & Carotenoids 2021 for
Orange Versus Commercial Conventional and Organic Yellow Hybrids
Independently Grown Multiple Indiana Locations by Tech Services, Inc (TSI)

	Yield bu/ac	Locations	Carotenoids
***GMO Hybrids** (avg. 4)	**219.8**	**6**	**18**
***Organic Hybrids** (avg. 4)	**200.7**	**6**	**17**
DH16 x ExPVP Dk B73	**203.0**	**4**	**37**
DH10 x ExPVP Dk Oh43	**201.2**	**2**	**43**
DH10 x ExPVP Dk B73	**195.7**	**4**	**37**
DH12 x ExPVP Holden's NSS	**198.4**	**4**	**40**
DH12 x ExPVP Pioneer B73	**185.7**	**5**	**41**

- Shows Yields of Top Two Hybrids are competitive with Organic Hybrids, and 92% of Top Current Commercial GMO Hybrids

- Yields are Very Promising
- Large Pipeline of Double Haploid Inbreds from Orange Population and Orange x Yellow exPVP lineages
- Diversifying into Orange Popcorn and Sweet Corn
- Combinations of Different Considerations:

  Expansion and Yield and for Popcorn,

  Taste and Appearance for Sweet Corn

Orange Popcorn

- **Low Calorie**
- **Whole Grain**
- **High in Fiber**
- **Non-GMO**

Orange Popcorn

- **More Nutritious**
- **Natural "Butter" Color**

Early Generation Orange Popcorn Conversion

Segregation on ear

We Select Most Orange Kernels to Plant

It works well, sometimes better, faster, cheaper than molecular markers….

Pedigree	Expansion	Expan Rank	Yield Mean	Yield Rank
Commercial Popcorn Hybrid 3	45.5	1	7621	3
Commercial Popcorn Hybrid 1	44.3	3	7324	10
Com. Hybrid 2 – Converting to Orange	45.0	2	7147	13
Experimental Orange Testcross Hybrids	42.5	6	7612	5
(BC3 or BC2F2 to One Parent) X (Other)	40.0	20	7984	2
	38.5	27	8141	1
	41.5	13	7435	8
	42.8	4	7024	17

Must have Good Expansion and Good Yield

Making Good Progress

- **Commercial Popcorn kernels 10-12 ug/g Lutein and Zeaxanthin before popping, after popping 5-6 ug/g L /Z**
- **Orange Popcorn 30 ug/g Lutein and Zeaxanthin before popping**
- **30ug/g x 50% Popping Destruction = 15 ug/g x 100g = 1500 ug**

  or 1.5 mg L/Z serving. We should eat 4-8 mg per day of L/Z for

  good visual health
- **Making Good Progress on Increasing Carotenoids. Two new lineages show approximately 40 ug/g Lutein and Zeaxanthin, and high 40s for total carotenoids which important for providing orange color**

- Advanced Breeding Line on Left

Ear F1 Seed just after harvest below

After Popping Carotenoids are 2X to 2.5X higher than Commercial Popcorn

Initial Proof of Concept Efforts in Sweet Corn Encouraging

Day 21 after pollination

Day 21 Post Pollination - Fresh Eating Stage for Sweet Corn
Commercial Sweet Corn Hybrid vs Orange Flinty Dent Experimental LIne

Levels of Zeaxanthin + Lutein, Total Carotenoids (mg/100g) fresh weight eating stage sweet corn kernels (21 DAP) - much Higher than anticipated !

*

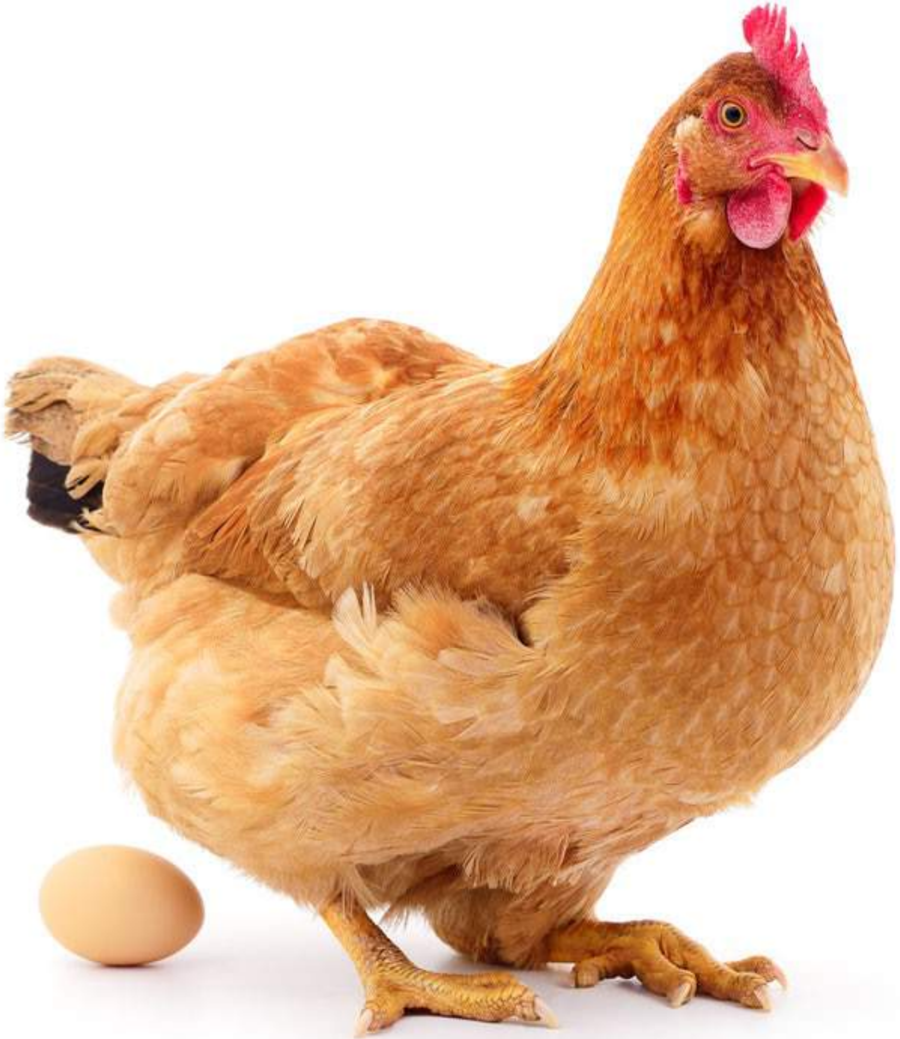
Line	% Orange	% Sweet	Lutein + Zeaxanthin		Total Carotenoids
Best Orange Field (1)	100%	0%	5.3		5.9
Orange x Sweet (4)	50%	50%	3.1 – 6.8		3.7-8.1
Orange x Sweet (4) Greenhouse	25%	75%	1.3-2.5		2.6-3.6
Sweet Bicolor (2)	0%	100%	0.5-1.6		0.5-1.7
Sweet White (1)	0%	100%	0.28		0.31

Some Primary Dietary Sources of Lutein and Zeaxanthin (L/Z) Souce: USDA Food Data Central*

Note: Carotenoids in Kale less Bioavailable than in Corn

Source		Kale	Peas	Yellow Sweet Corn	Orange Sweet Corn Projected Estimates**
L/Z Level (mg/100g)		5-6.3	1.7-2.4	0.7-1.0	3 - 6
Per Cup Equivalent (variable g)		4.2-5.6	2.9-3.8	1.3-1.7	5 - 10

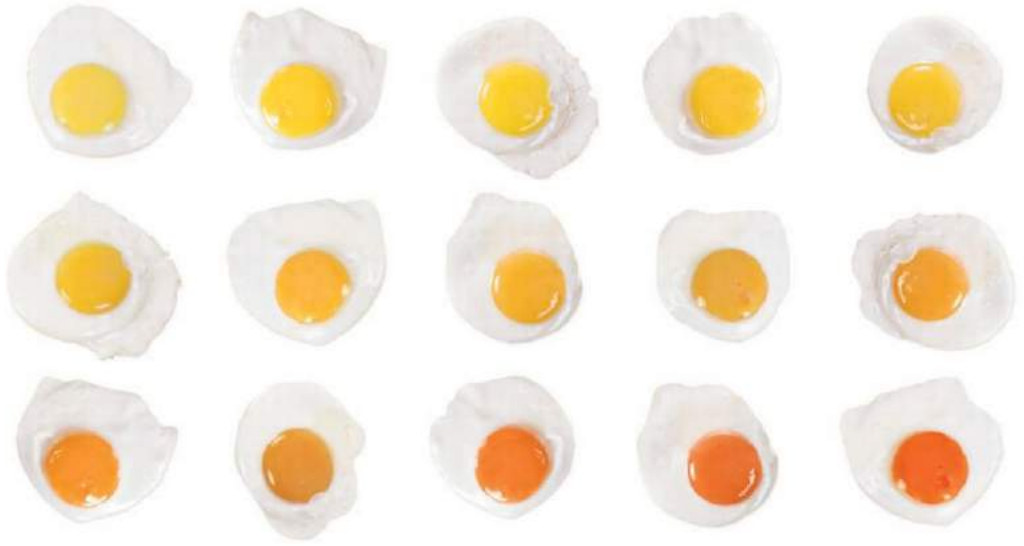
***Orange Sweet Corn values calculated based on NutraMaize estimates.* *Have become aware other estimates vary a lot!

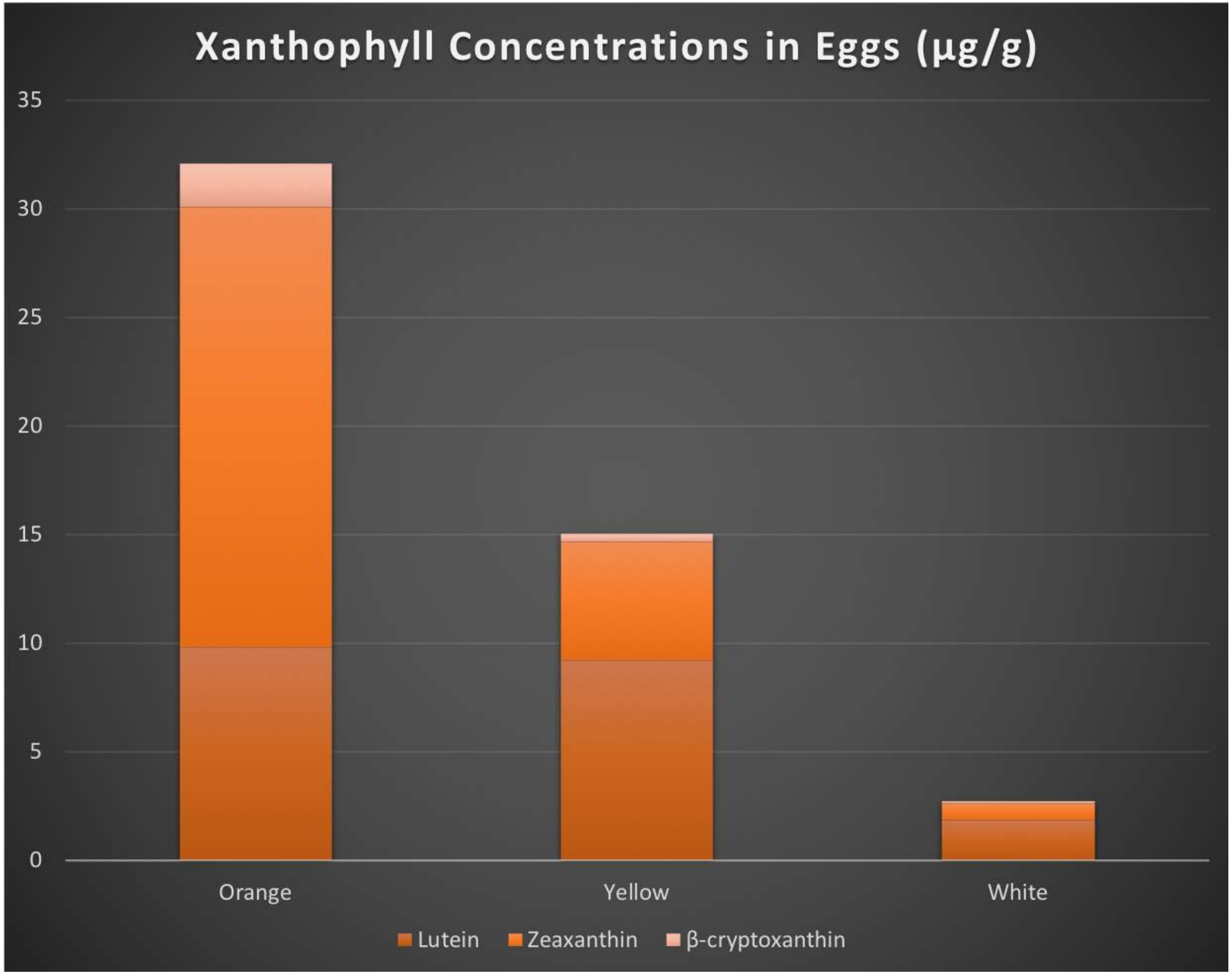


Supported by USDA NIFA SBIR to NutraMaize and subaward to Purdue

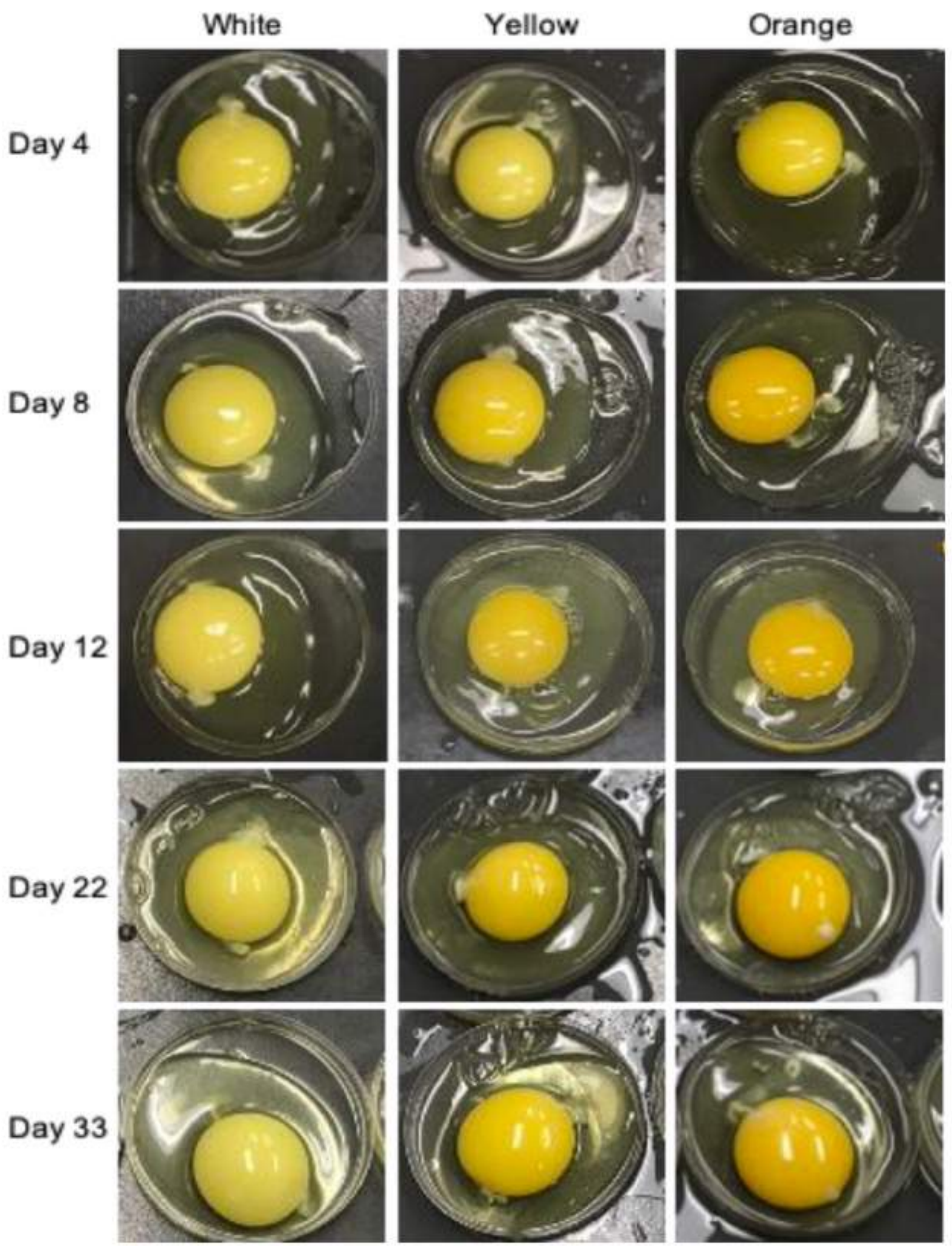
Laying Hens, Eggs & Carotenoids

- 300 million laying hens in USA alone
- Produce 7+ billion table eggs per month
- Color comes from Xanthophyll carotenoids
- Egg yolk carotenoids 2x more bioavailable than from spinach
- High per capita consumption (270 eggs per year)

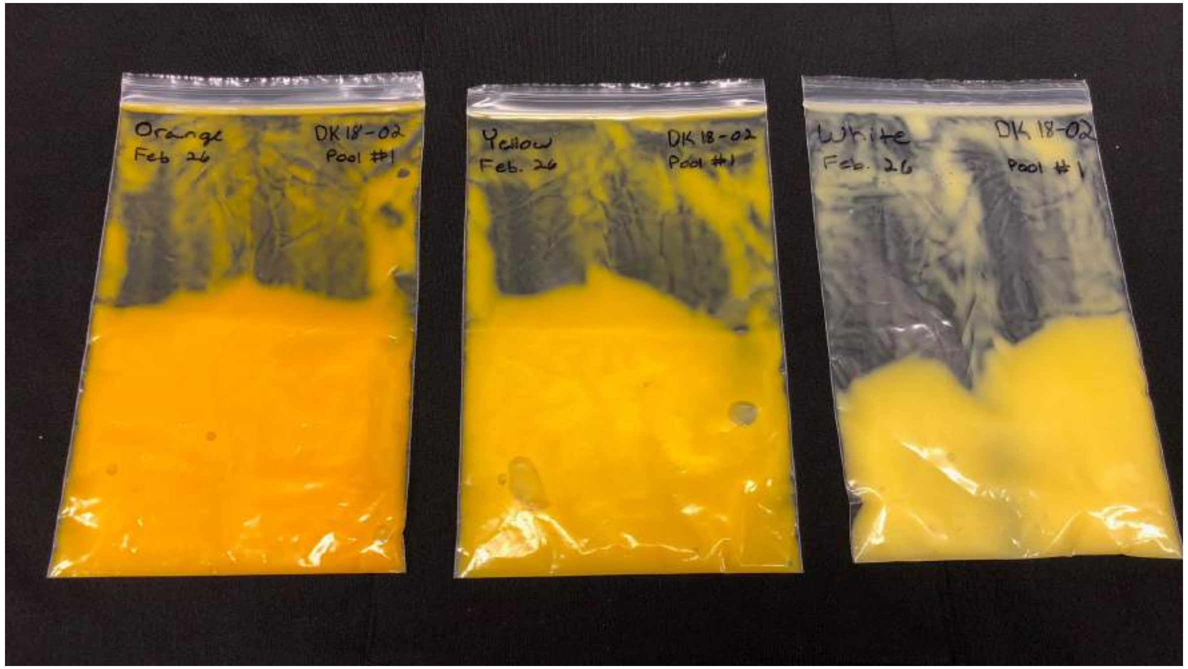




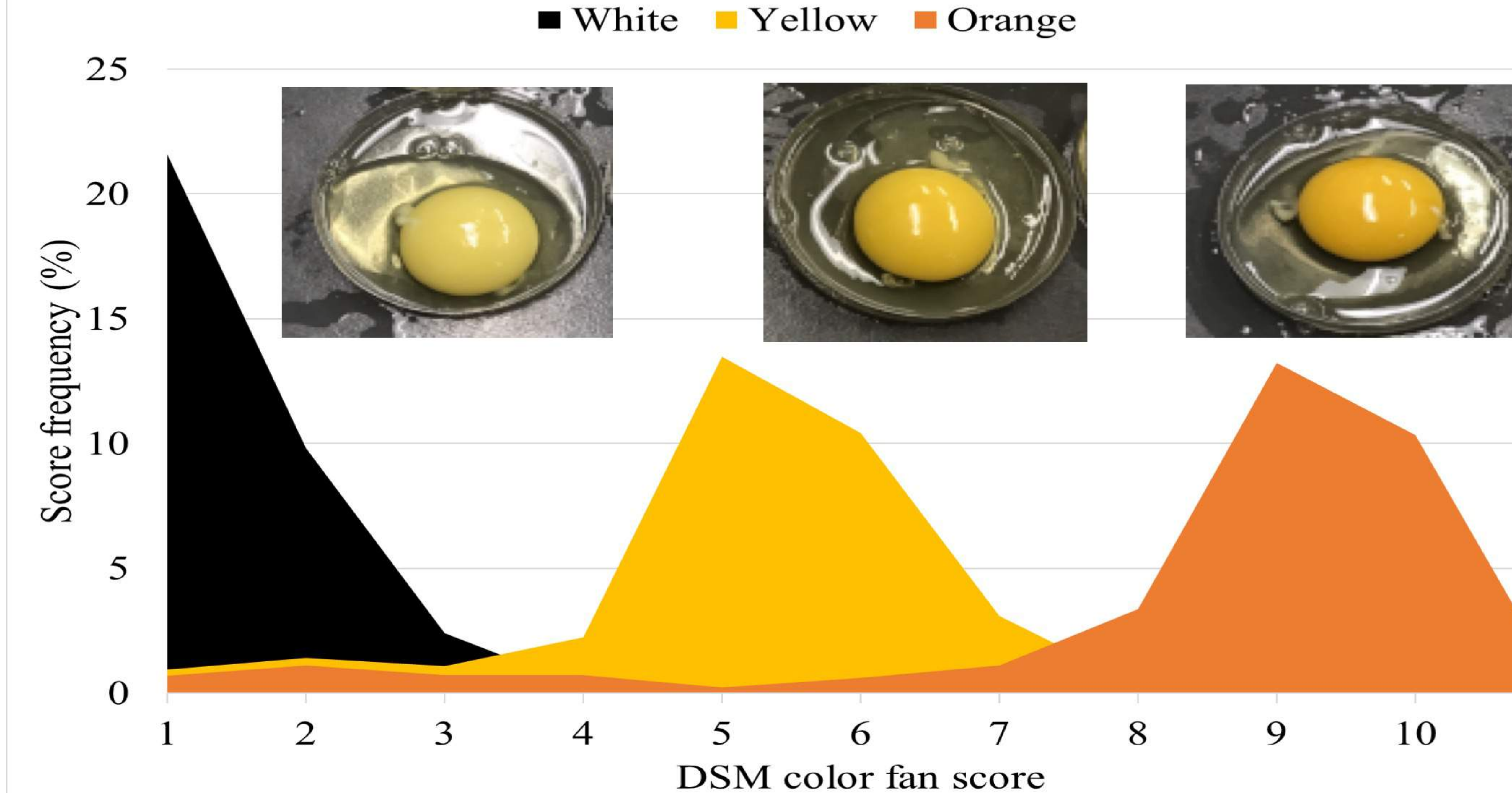
From Published Study at ASREC – Only Difference in Feed was Type of Corn – Orange, Yellow, White



Ortiz, D. et al., Poultry Science 101117 (2021).



Bulk of eggs from diets with orange, yellow and white corn



Some High Quality Egg Producers would like a ***<u>Consistent</u>*** Fan Score of about 8. As 9 or 10 may be too High

What's in an Egg from Hen Fed Orange Corn ?

Orange Corn diet produced eggs with higher DSM yolk color score, 6 to 10, compared to yellow diet, 5 to 6 DSM ($P < 0.01$)

Color is what matters for most egg producers In USA. They want it around DSM 8 in USA - orange but not too high and very orange.

Orange Corn diet produced eggs with total xanthophylls 23.5 to 35.3 µg/g of egg yolk compared to the yellow diet 12.3 to 17.7 µg/g xanthophylls

18-20g for an egg yolk, generally about 30% of the weight of the egg. A score of DSM 8 is maybe about 29 ug/g x 18g = 232 ug or about 0.2 mg xanthophylls per egg. 0.4 mg for two eggs

Super Egg maybe 22g x 35ug/g = 770 ug / 0.75 mg per egg, 1.5 mg two eggs ?

Yellow Orange White

Carotenoids are Lipid/Fat Soluble. Chicken Paws and Footpads Have Fat and Accumulate Antioxidant Carotenoids
Poultry Science 2021 May; 100(5): 101054.

Foot Pad Dermatitis - Causes Significant Economic Losses

Feeding Orange Corn Reduced FootPad Dermatitis

More Prevalent in Cage Free as Walking on Feces and Urine....

Mechanism presently unknown. Carotenoids are antioxidants, lipid soluble, accumulate in fat in body and footpads. Carotenoids involved in human immune system.

Footpad Dermatitis very high frequency in Organic Systems

Organic Egg Producers account for 4.5% of Total Egg Production in USA = 13.4 Million Laying Hens that Consume 600,000 Tons of Feed a year.

If NutraMaize's Orange Corn were to provide only 5% of the feed required by Organic Laying Hens, the Sale of Seed alone would produce over $2 Million in Revenue.

Orange Corn is higher in protein, and may replace in diets some organic soybean meal which is 4X $

Relevant Funding for Startup Enterprises:

NSF Small Business Technology Transfer (STTR) Grant Program, Phase I and II

USDA NIFA Small Business Innovation Research (SBIR) Program, Phase I and II

Acknowledgements

Many, many fine souls and organizations that have worked together……..

- CIMMYT – IITA – USAID – HarvestPlus+
- NSF Plant Genome Research Program – NSF Small Business Technology Transfer (STTR) Program – USDA Small Business Innovation Research (SBIR) Program
- Patterson Endowed Chair, Other Sources…..

This is in Zambia, yet in my view – The Entire World ‘Should’ Look Like This

Orange Corn and Golden Rice

- Golden Rice has just completed two cycles of production in Philippines.

Both for seed increase and some for for milling for local people to try.

- Higher ProVitaminA Orange Corn been growing and being consumed in Africa for about ten years......

Results from Nigeria show some good consumption.

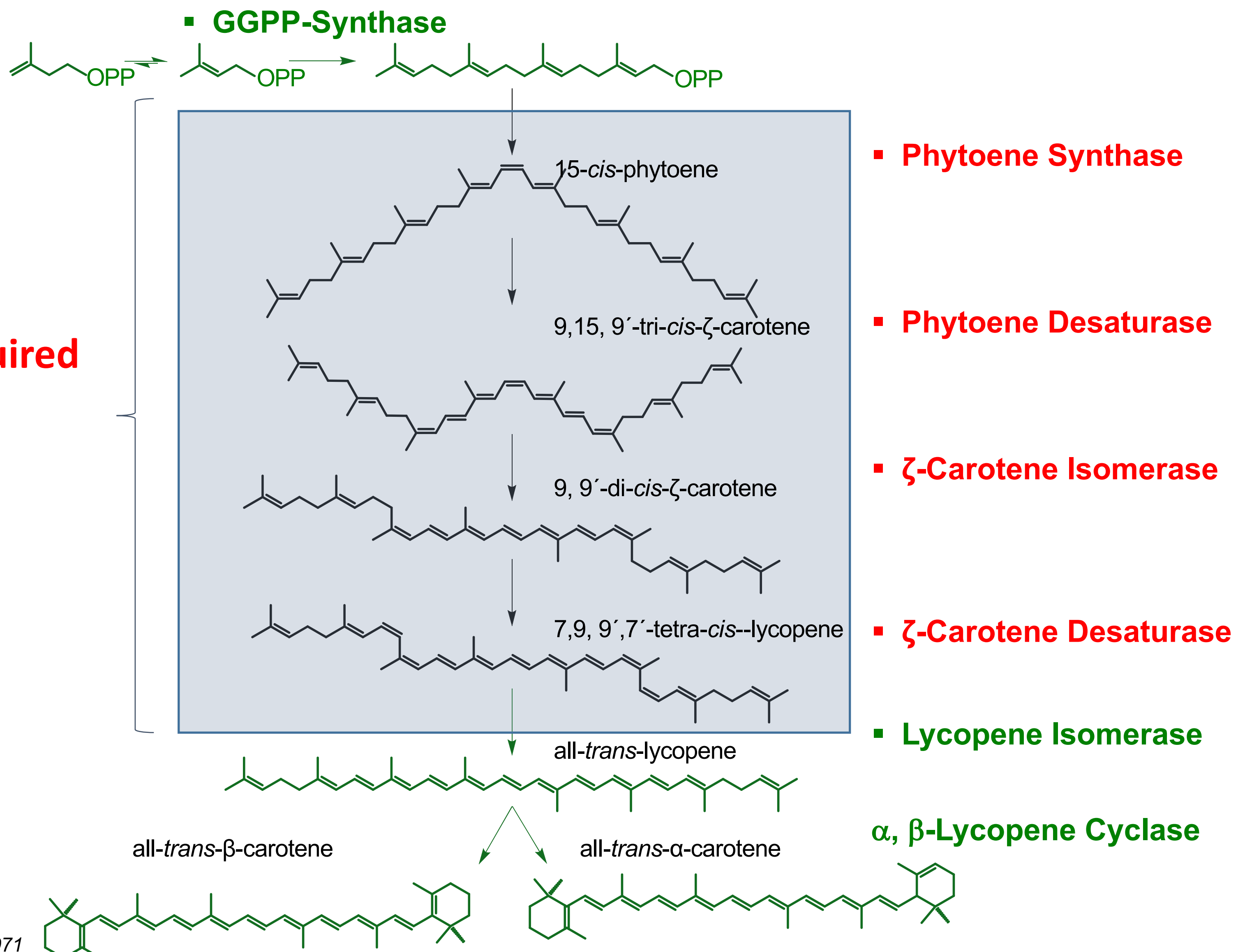

Golden Rice and other traits cannot be bred
…Engineering is necessary
Breeding where possible
Genetic engineering where required

The Golden Rice Principle: Bridging a gap

Wild-type endosperm

„Absent“
2 genes are required to bridge

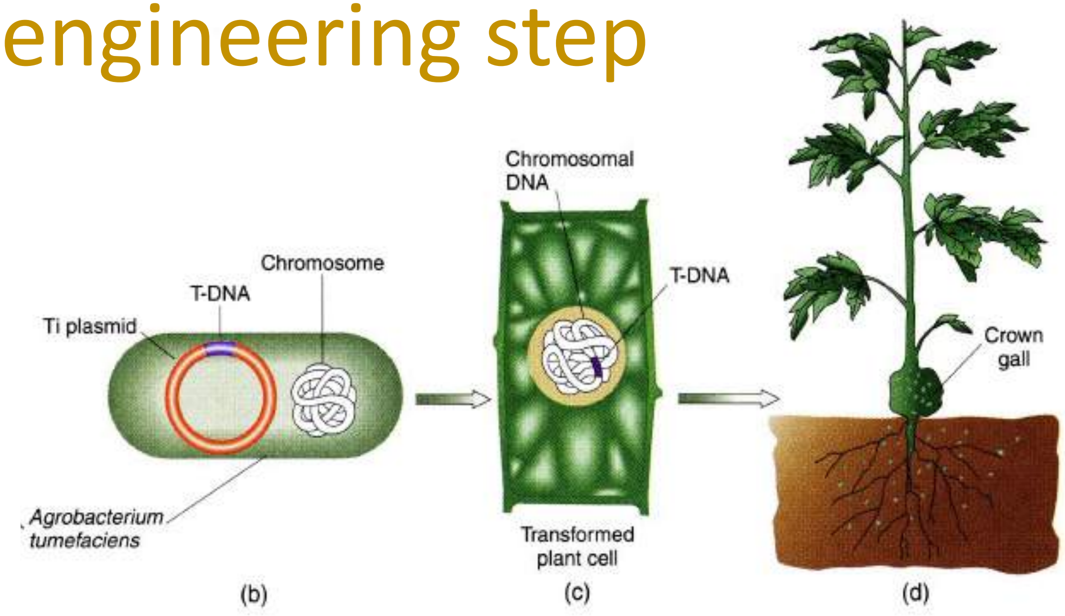
Wild-type endosperm



Provitamin A

Burkhardt et al., (1997) Plant J. 1, 1071
Schaub et al. (2005), Plant Physiol. 138: 441

The engineering step



The principle

The construct

Transformation

The process

Multiplying the efficiency and impact of biofortification through metabolic engineering of rice for the Philippines

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Nutrition-Smart Agricultural Strategies

- **Food Staples** – Increase **Density** of Nutrients
  - Biofortification
  - Fertilizers and sprays
  - No extra cost to consumers
- **Non-Staple Foods** – Increase **Quantities**
  - Milk, Eggs, Small Fish, Specific Green Leafy Vegetables
  - Home gardens, hybrid seeds (multiple nutritious foods)
  - Requires higher incomes and/or lower food prices

Which Foods Are Sources of Minerals & Vitamins?

- The Food and Nutrition Research Institute (FNRI) has conducted periodic nationwide dietary surveys since the 1970's. The FNRI publishes estimates of food intakes for 164 individual foods.
- Of the 164 individual foods, which **single food** contributes by far the most minerals, vitamins, and other nutrients than any other single food to Filipino diets?

Point 1: Rice Provides a Significant Base of Nutrient Intakes

Percent Contribution of Milled Rice to Nutrient Intakes in the Philippines, 2015

Nutrient	Percent Contribution	Nutrient	Percent Contribution
Energy	59%	Protein	40%
Carbohydrates	76%	10 of 11 Amino Acids	30-40%
Calcium	20%	Vitamin A	0%
Copper	32%	Vitamin C	0%
Iron	33%	Thiamine (B1)	39%
Magnesium	38%	Riboflavin (B2)	20%
Manganese	53%	Niacin (B3)	45%
Phosphorous	33%	Vitamin B5	57%
Potassium	15%	Vitamin B6	32%
Zinc	41%	Folate (B9)	10%

False

- Rice and other food staples lack minerals and vitamins

True

- Rice and other food staples are **not dense** in minerals and vitamins
- The significant contribution of food staples to Filipino **dietary quality** is due to the high volume of consumption
- Filipinos of all income groups eat about the same amount of rice

Point 2: Rice Consumption Does Not Vary By Income

Per Capita Energy Intakes Per Day for Bangladesh and Philippines By Income Group

	Poorest	Middle	Richest
Food Staples	1816	1848	1876
Non-Staple Plant Food	339	427	474
Fish and Animal Foods	47	59	92
All Food Groups	2201	2334	2442

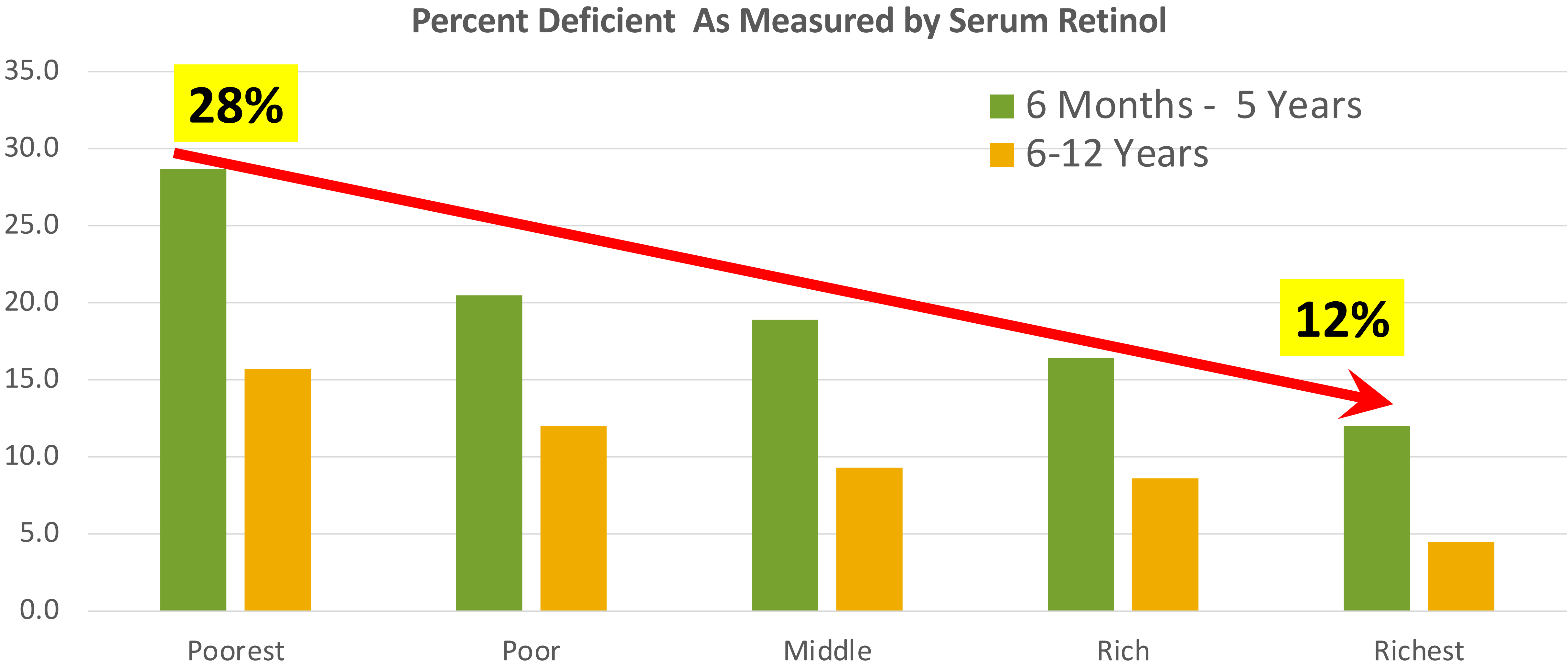
Bangladesh

	Poorest	Middle	Richest
Food Staples	1271	1254	1146
Non-Staple Plant Food	249	287	351
Fish and Animal Foods	137	261	465
All Food Groups	1657	1802	1962

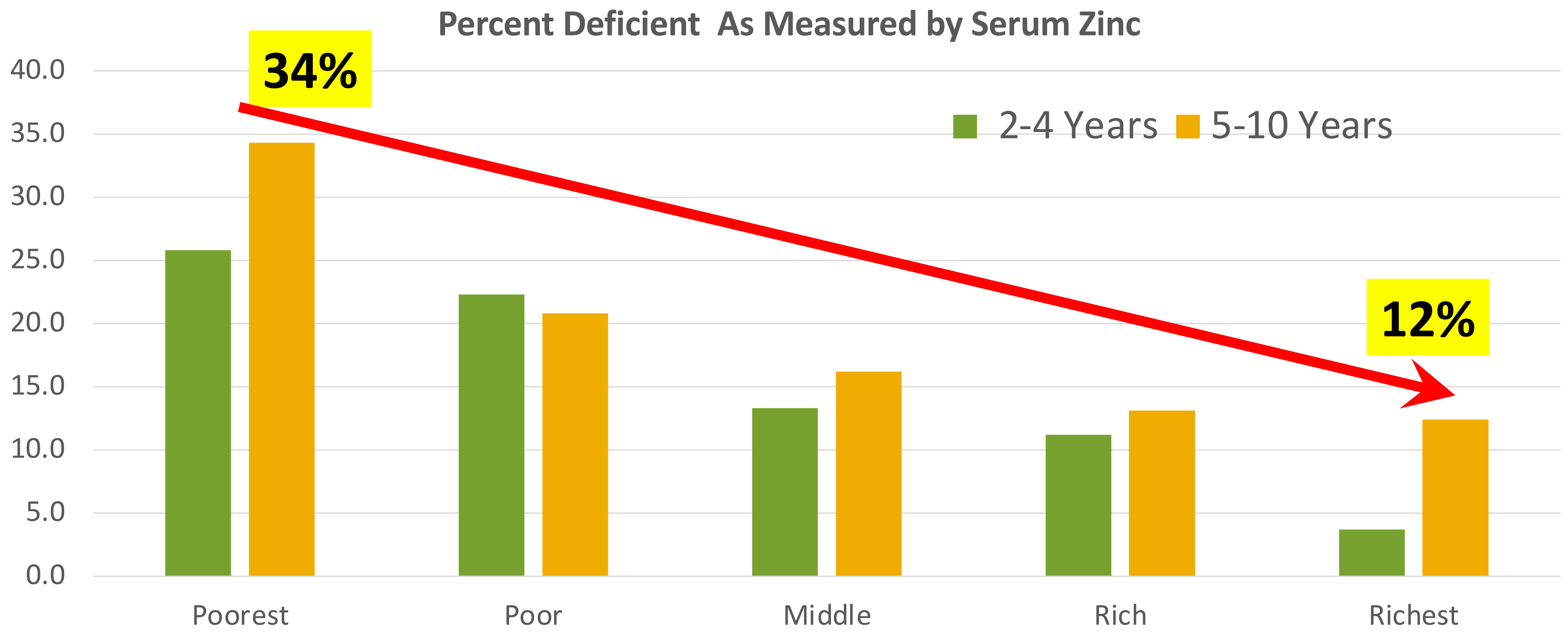
8th NNS (FNRI-DOST, 2013)

Philippines

Preschooler/Child Vitamin A Deficiency Is Strongly Related to Income, Philippines 2013



Preschooler/Child Zinc Deficiency Is Strongly Related to Income, Philippines 2013



Calculation of Retinol Activity Equivalent Per Gram for Transgenic Vitamin A in Rice

Density in harvested rice (Malusog 1 in Philippines)	14	ug/g milled	total carotenoids
Percent retention after two months of storage	40%		
Percent provitamin A carotenoids	80%		
Total provitamin A carotenoids density after 2 months	4.5	ug/g milled	total provitamin A carotenoids
Conversion to retinol equivalents	22.4%	(see calculation below)	
Density before cooking as purchased	1.0	ug/g retinol activity equivalents	

Calculation of bioavailability for women				
Conversion rate	betacarotene	3.8	Portion of total carotenoids	67%
Conversion rate	other provitamin A carotenoids	5.8	Portion of total carotenoids	33%
Weighted conversion rate		4.5		
Bioavailability percentage		22.4%		

Density of Provitamin A, Iron, and Zinc Measured in Milled Rice By Type of Variety

Nutrient	Conventionally-Bred	Transgenic	Increment
Provitamin A	0	1 (0.05)	**+1**
Zinc	11.6 (1.6)	43.2 (3.6)	**+31.6**
Iron	1.9 (0.8)	9.7 (0.7)	**+7.8**

Provitamin A = RAE per gram of milled rice before cooking
Iron and zinc = mg/kg

Increased Intake of Provitamin A, Iron, and Zinc in Milled Rice By Type of Variety By Wealth Quintile

Nutrient	Incre-ment Density	Milled Rice Intake Per Day	Increased Intake Per Day	Base Intake Average Total Pop	Base Intake Poorest Quintile
Provitamin A	+1	300 g	+300 RAE	507 RAE	329 RAE
Zinc	+31.6	300 g	+10.5 mg	7.4 mg	6.3 mg
Iron	+7.8	300 g	+2.3 mg	9.6 mg	8.4 mg

Provitamin A = RAE per gram of milled rice before cooking
Iron and zinc = mg/kg (density); mg/day (intake)

Use of Genetic Engineering Strategies: The Next Level in Increasing the Impact of Biofortification

As compared with conventional breeding:

- Faster path to increasing densities of multiple nutrients (thus far, biofortification has been limited to single nutrient additions)
- Densities of specific nutrients can be increased (e.g. Zn & Fe)
- Increasing density of specific nutrients is made feasible (e.g. provitamin A in rice)
- Climate-smart traits can be bundled with nutrient traits

Golden Rice "Plus" (provitamin A, iron, and zinc all in one rice variety) could be the breakthrough in demonstrating the power and usefulness of genetic engineering.