Biofortification of maize Progress and Perspectives

Natalia Palacios Rojas

n.palacios@cgiar.org

Thanda Dhliwayo, Felix San Vicente, Sudha Nair, Thokodzile Ndhela, Luis Narro, Xuecai Zhang, Aldo Rosales, Luisa Cabrera, Aide Molina,Yadhira Ortiz

Ciudad de Mexico, March 2018

MAIZE

HarvestPlus
Better Crops • Better Nutrition

CIMMYT

Sustainable Development goals

Land use

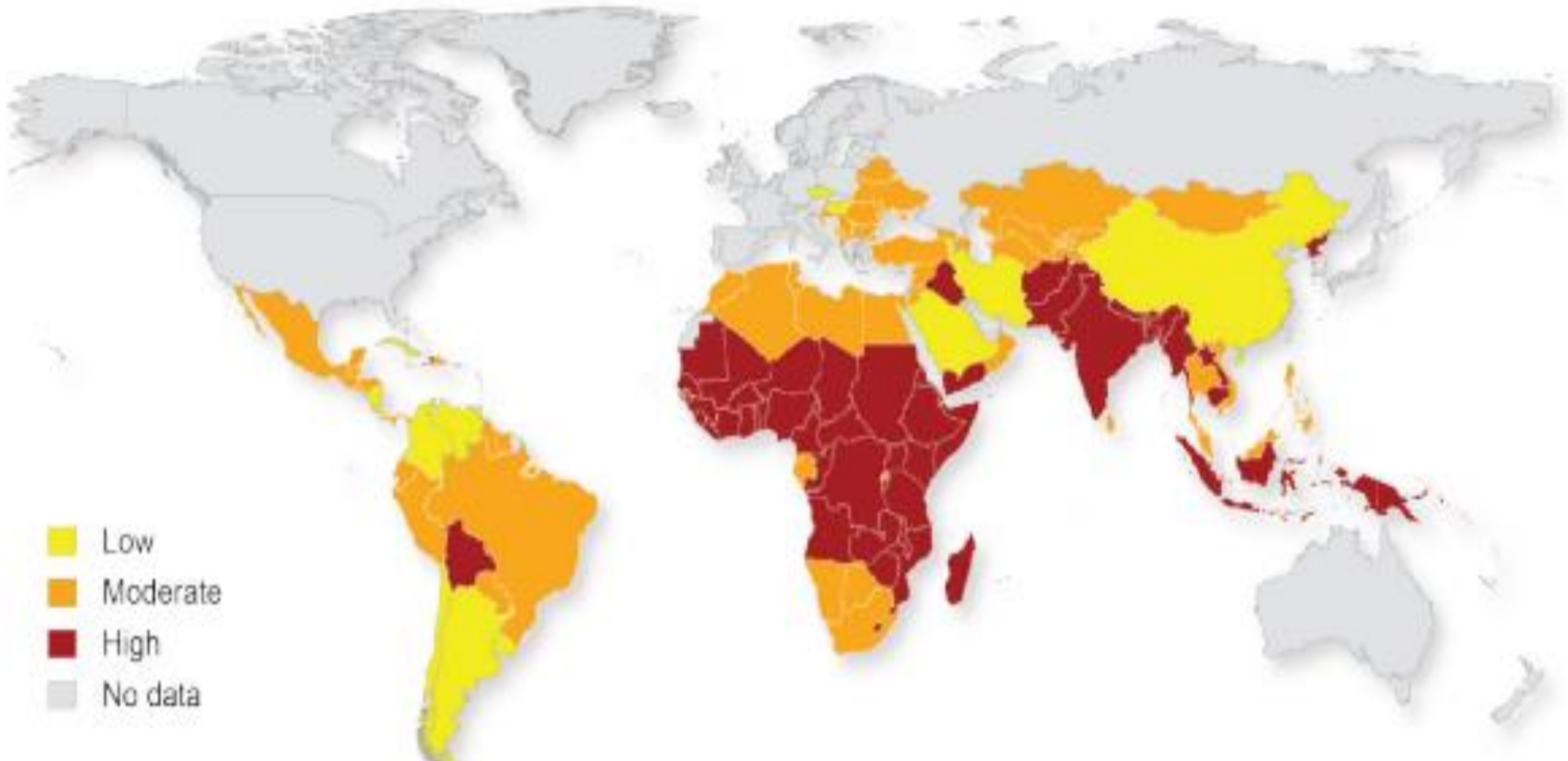
Natural resource management

Climate change

Rural transformation

Nutrition and health

Severity of Micronutrient Deficiencies: Vitamin A, Iron, and Zinc



Source: World Health Organization (WHO) children under 5 prevalence data

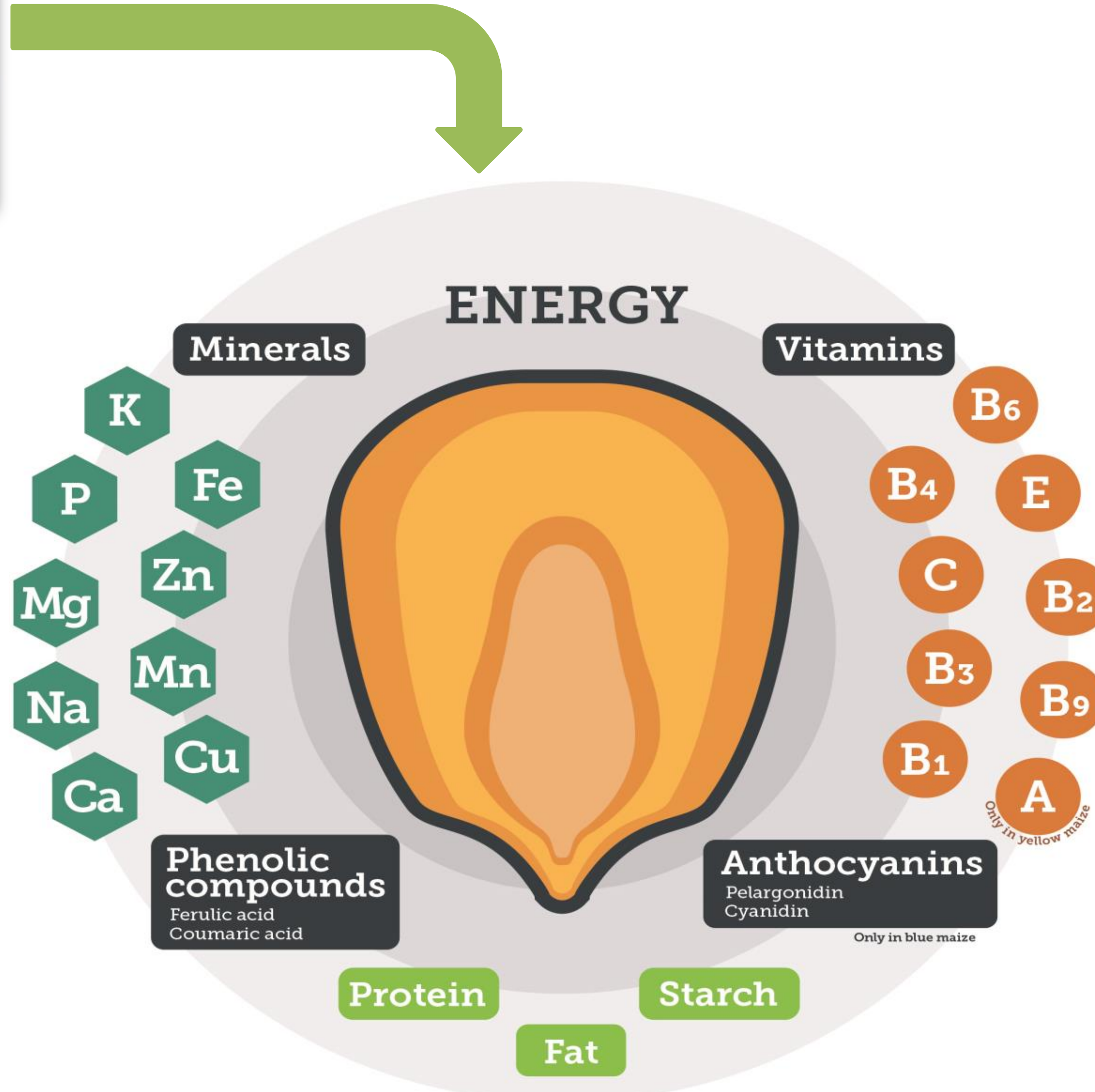
Maize and wheat: what the world eats



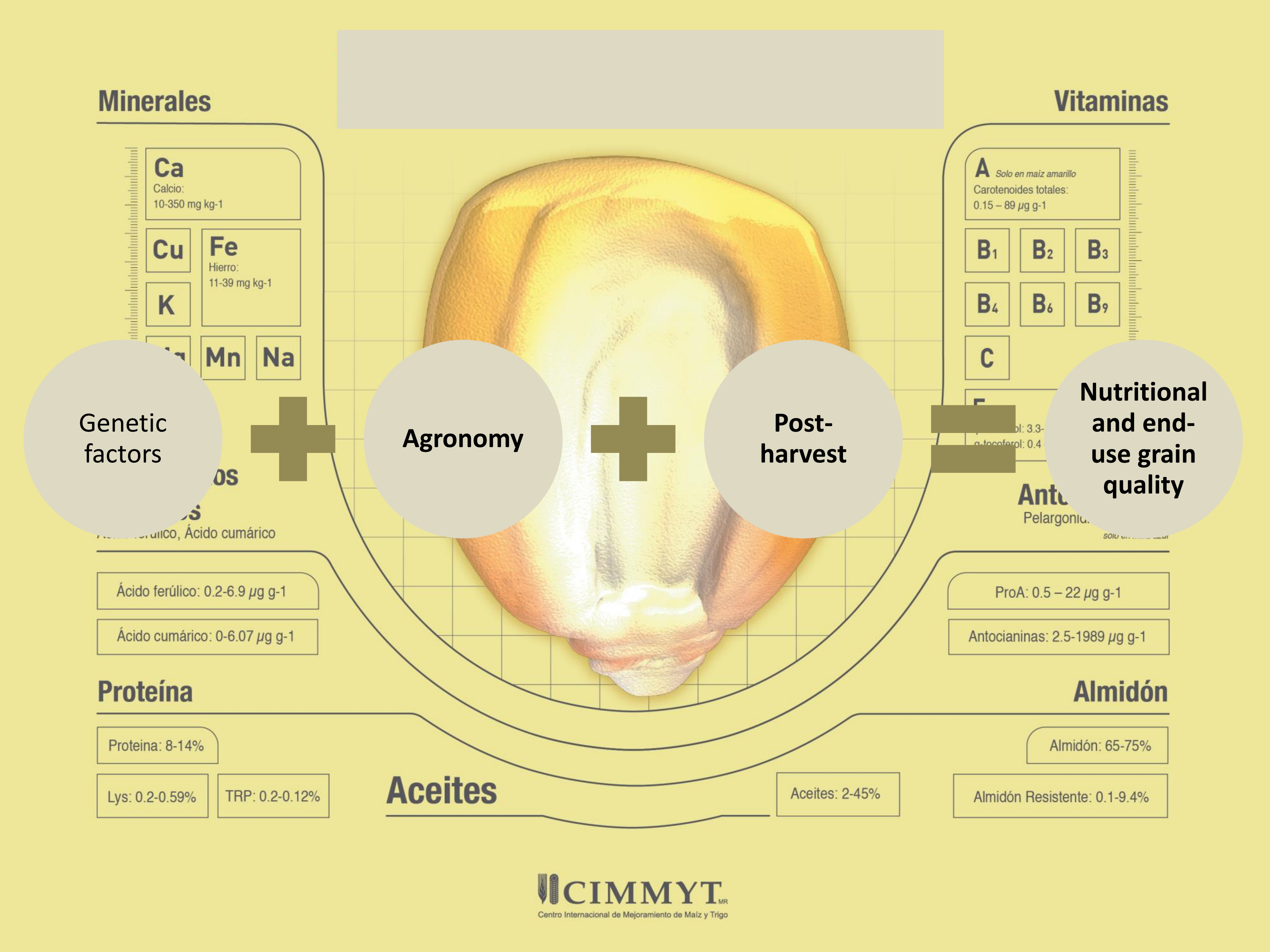






ENERGY
Minerals
K
P
Fe
Zn
Mg
Mn
Na
Cu
Ca
Vitamins
B6
B4
E
C
B2
B3
B9
B1
A
Only in yellow maize
Phenolic compounds
Ferulic acid
Coumaric acid
Anthocyanins
Pelargonidin
Cyanidin
Only in blue maize
Protein
Starch
Fat


Genetic factors
+
Agronomy
+
Post-harvest
=
Nutritional and end-use grain quality
Minerales
Ca
Calcio:
10-350 mg kg-1
Cu
Fe
Hierro:
11-39 mg kg-1
K
Mn
Na
Vitaminas
A Solo en maíz amarillo
Carotenoides totales:
0.15 – 89 µg g-1
B1 B2 B3
B4 B6 B9
C
Ácido ferúlico: 0.2-6.9 µg g-1
Ácido cumárico: 0-6.07 µg g-1
ProA: 0.5 – 22 µg g-1
Antocianinas: 2.5-1989 µg g-1
Proteína
Proteina: 8-14%
Lys: 0.2-0.59%
TRP: 0.2-0.12%
Aceites
Aceites: 2-45%
Almidón
Almidón: 65-75%
Almidón Resistente: 0.1-9.4%
CIMMYT
Centro Internacional de Mejoramiento de Maíz y Trigo

Maize nutritional benefits

Biofortification

- QPM
- ProVA
- Zn

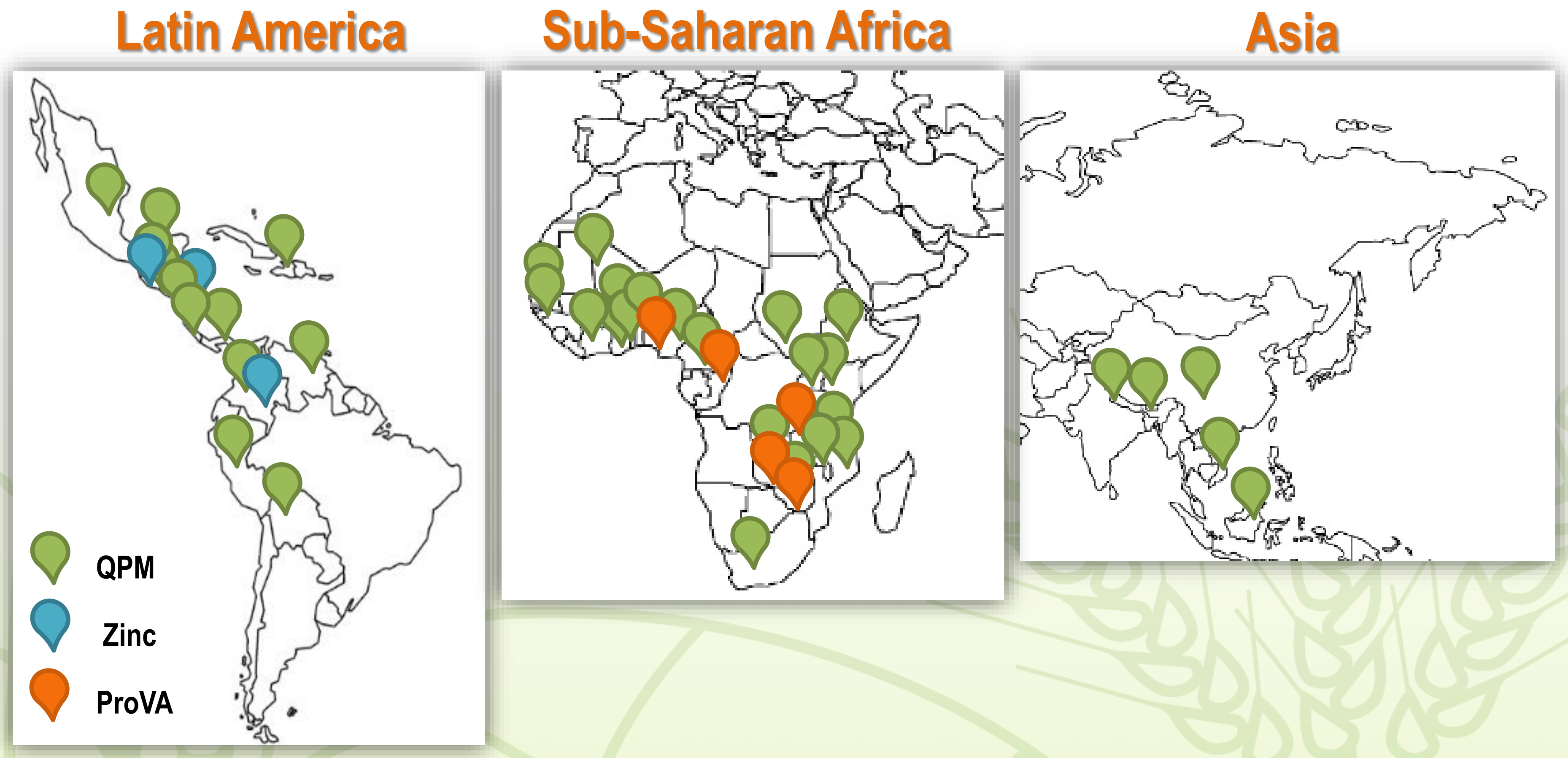
Processing

- Nixtamalization
- Fermentation

Functional food

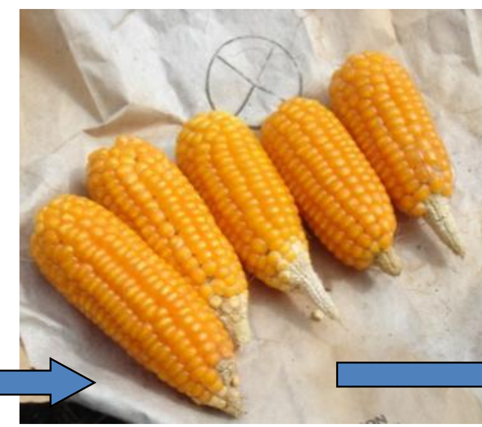
- Fortification
- Eggs high in ProVA

QPM, pro A and zinc varieties released in the world



Provitamin A-enriched Maize

2004: First crosses between White elite lines by Provitmin A sources (not elite)

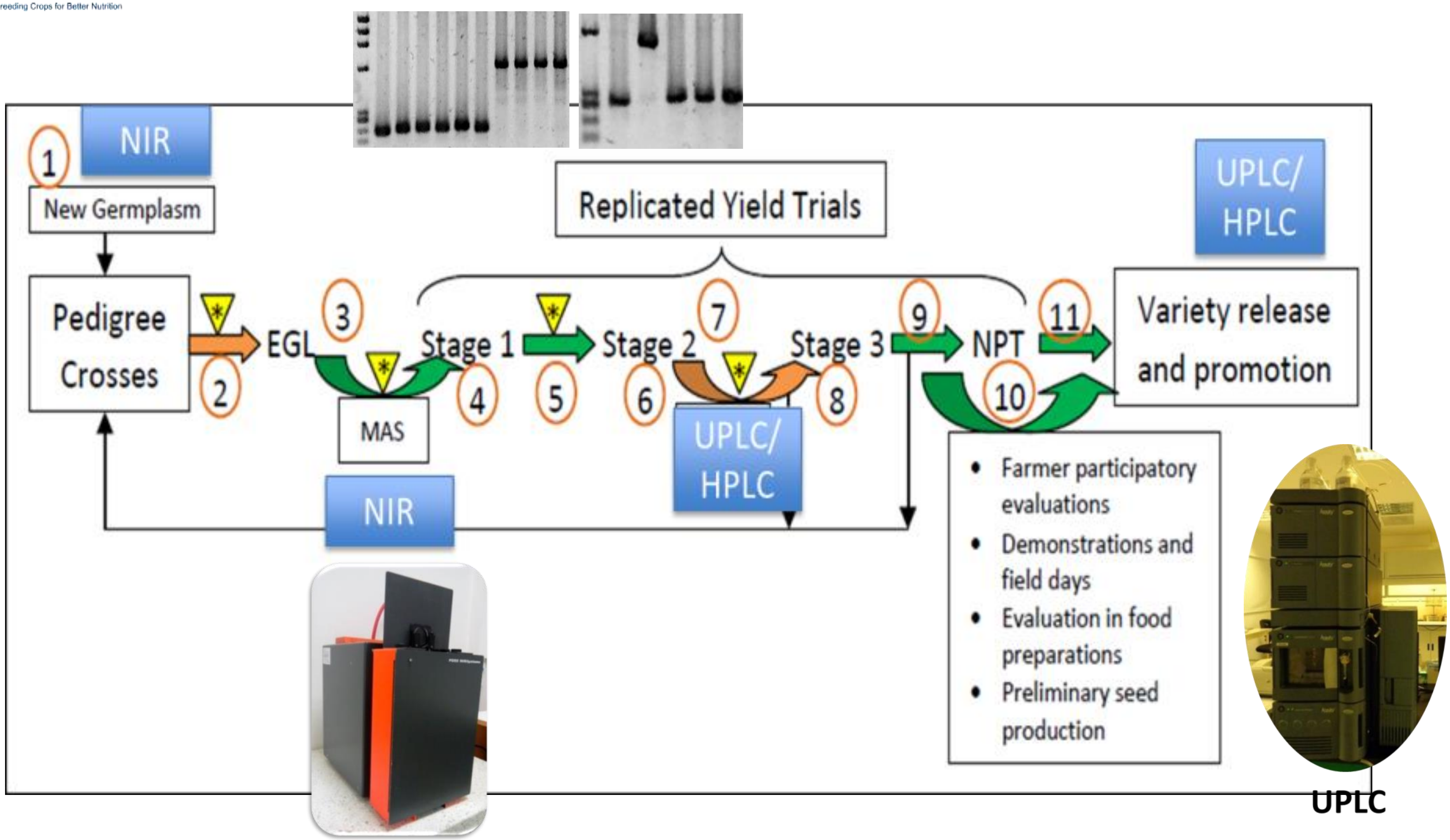


Menkir, A. et al. (2018) Vitamin A-Biofortified Maize:
Exploiting Native Genetic Variation for Nutrient Enrichment. Science Brief: Biofortification No. 2 (February 2018).

Continuous breeding for last 12 years….

- Improved germplasm
- Molecular markers for high BC
- DH lines
- Screening tools
- Breeding methods
- Product deployment

HarvestPlus
Breeding Crops for Better Nutrition


NIR
1
New Germplasm
Pedigree Crosses
2
EGL
3
MAS
NIR
Replicated Yield Trials
Stage 1
4
5
Stage 2
6
7
UPLC/ HPLC
Stage 3
8
9
NPT
10
11
Variety release and promotion
UPLC/ HPLC
Farmer participatory evaluations
Demonstrations and field days
Evaluation in food preparations
Preliminary seed production
UPLC

Possible to develop elite maize hybrids with high grain yield and Provitamin A

Table 6. Pearson phenotypic correlation coefficients (top) and *P* values (bottom) among agronomic traits and carotenoid concentrations (154 df). GY, grain yield; AD, anthesis date; PH, plant height; LUT, Lutein; ZEA, Zeaxanthin; βCX, β-cryptoxanthin; βC, β-carotene; PROA, total provitamin A; GD, shared allele genetic distance.

	GY	AD	PH	LUT	ZEA	βCX	βC	PROA†
AD	0.06							
	0.42							
PH	0.51	0.32						
	<0.01	<0.01						
LUT	−0.04	−0.19	0.10					
	0.59	0.02	0.23					
ZEA	−0.01	−0.07	0.05	0.52				
	0.94	0.39	0.54	<0.01				
BCX	0.22	0.43	0.31	0.28	0.35			
	0.01	<0.01	<0.01	0.00	<0.01			
BC	−0.09	−0.18	−0.15	−0.16	0.09	−0.16		
	0.27	0.03	0.07	0.05	0.28	0.05		
PROA	0.00	−0.05	−0.05	−0.07	0.10	0.09	0.93	
	0.97	0.53	0.56	0.39	0.21	0.25	<0.01	
GD	0.37	−0.30	0.18	0.05	0.18	−0.02	0.03	0.03
	<0.01	0.00	0.02	0.50	0.03	0.79	0.74	0.74

†Total provitamin A concentration = all-trans β-carotene + 9-cis β-carotene + 13-cis β-carotene + 0.5(β-cryptoxanthin).

Grain yield is NOT significantly correlated with Provitamin A concentration (= no correlated positive or negative selection)

(Egesel et al. Crop Sci. 2003;
Suwarno et al. Crop Sci. 2014)




Maize-based food product	Process	% PVA retention	Reference
Popcorn	Heating	70 to 80%	unpublished
Boiled corn	Boiling	70%	Alamu et al., 2015
Roasted corn with husk	Roasting	>100%	L. Cabrera (submittedd)
Roasted corn without husk	Roasting	80-90%	Alamu et al, 2015; Barbosa et al., 2015
Samp	De-hulling, cooking	53-98%	Mugode et al., 2014
Nshima (thick porridge) and thin porridge	Hammer milling, cooking	70-80%	Taleon et al., 2017
Nixtamalized tortilla (Lime-cooked maize flat bread)	Traditional nixtamalization (cooking, steeping, milling, dry cooking)	80- >100%	Rosales et al., 2016
Nixtamalized tortilla (Lime-cooked maize flat bread)	Milling, nixtamalization by extrusion, dry cooking	85-95%	Rosales et al., 2016
Nixtamalized chips (lime-cooked chips)	Traditional nixtamalization and frying	60-65%	Lozano-Alejo, 2007
Nixtamalized tamales (wrapped steamed lime-cooked dough)	Traditional nixtamalization, stone milling and steaming.	60%	unpublished





JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Biofortified Orange Maize Enhances β-Cryptoxanthin Concentrations in Egg Yolks of Laying Hens Better than Tangerine Peel Fortificant

Emily K. Heying,[†] Jacob P. Tanumihardjo,[†] Vedran Vasic,[†] Mark Cook,[†,‡] Natalia Palacios-Rojas,[§] and Sherry A. Tanumihardjo*[,†]

[†]Interdepartmental Graduate Program in Nutritional Sciences, Department of Nutritional Sciences, [‡]Department of Animal Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706, United States

[§]International Maize and Wheat Improvement Center (CIMMYT), Texcoco 56237, Mexico

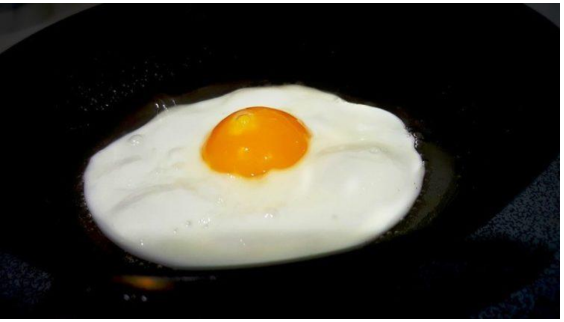
Biofortified maize can further enrich the poultry sector, which is one of the major drivers for maize demand in Asia and Latin America

Carotenoid source	ProVA maize	Tangerine	Yellow maize	White maize
16 Days of feeding	3.49 ± 1.9	3.22 ± 0.4	2.44 ± 0.2	1.14 ± 0.2
50 Days of feeding	**8.82 ± 1.0**	3.74 ± 0.5	1.93 ± 0.2	1.55 ± 0.7

Yolk from orange maize-fed hen   Yolk from white maize-fed hen

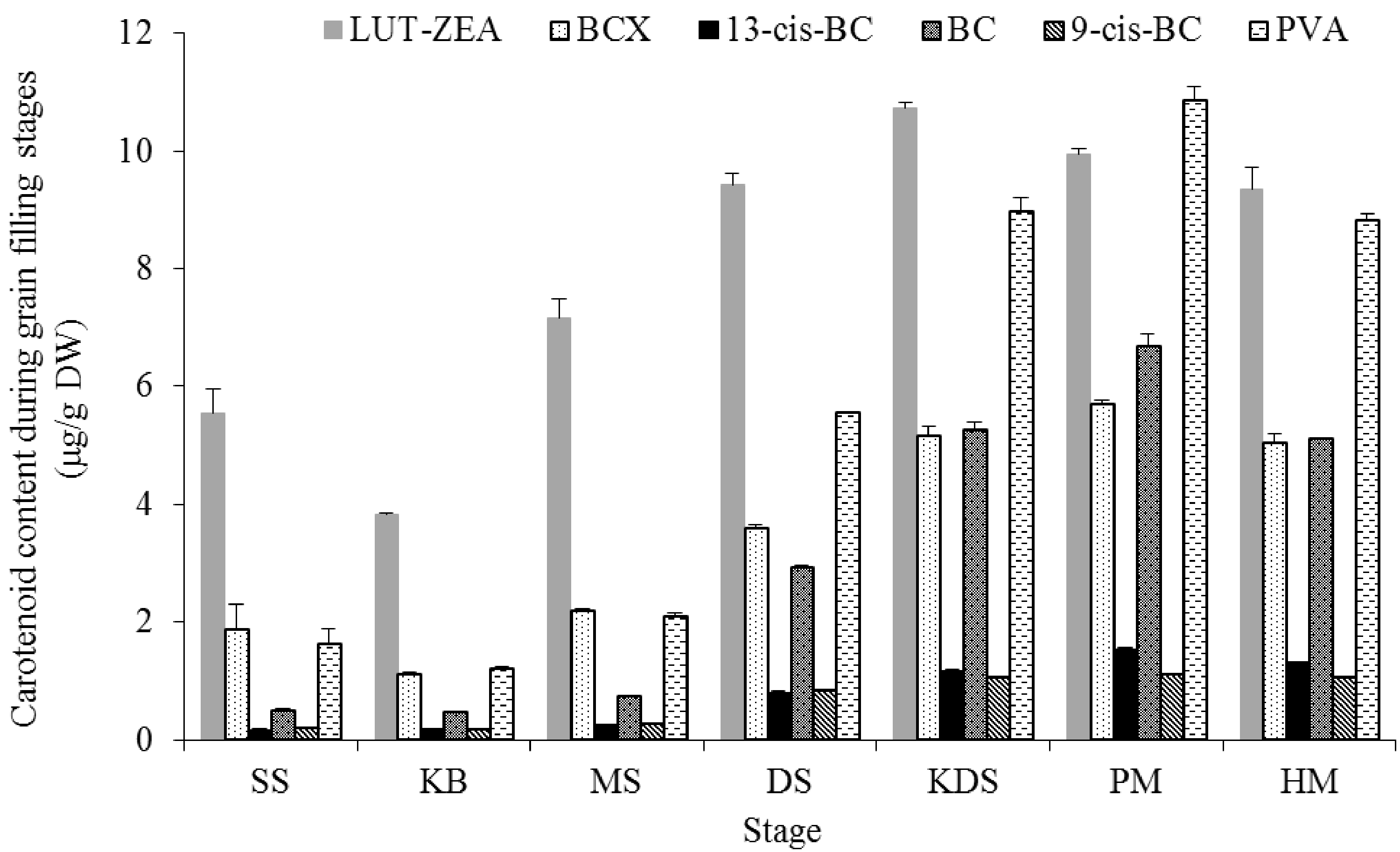
Retention of carotenoids in BCX-enhanced eggs after household cooking

hen diet	cooking method	β-cryptoxanthin
tangerine peel	raw	100
	microwaved	80.2 ± 9.3^{c}
	hard boiled	85.7 ± 12.3^{bc}
	fried	90.0 ± 15.6^{abc}
	scrambled	84.1 ± 15.7^{c}
biofortified maize	raw	100
	microwaved	97.5 ± 8.5^{ab}
	hard boiled	97.4 ± 5.2^{ab}
	fried	102 ± 6.2^{a}
	scrambled	84.0 ± 7.1^{c}



Sowa et al., 2017

Carotenoid content during grain filling stages for PVA hybrids



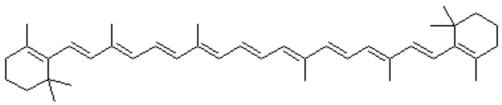
LUT-ZEA = lutein-zeaxanthin; BCX = b-criptoxanthin; 13-*cis*-BC = 13-*cis*-b-carotene; BC = b-carotene; 9-*cis*-BC = 9-*cis*-b-carotene; PVA = provitamin A carotenoids; SS = silking stage; KB = kernel blister; MS = milk stage; DS = dough stage; KDS = kernel dent stage; PM = physiological maturity; HM = harvest maturity. The symbol * indicate a significant difference ($P < 0.05$) for the carotenoid content throughout the kernel development stages.

Cabrera et al., 2018 submitted

Carotenoid losses: catabolic enzymatic and non-enzymatic degradation

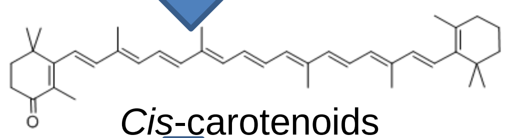
Oxidation, isomerization
NCEDs/CCDs

All-*trans*-carotenoids



Acids, light, heat

Oxygen, light, heat, metals, enzymes, pro-oxidants



Cis-carotenoids

Oxygen, light, heat, metals, enzymes, pro-oxidants

Epoxycarotenoids

Apocarotenoids

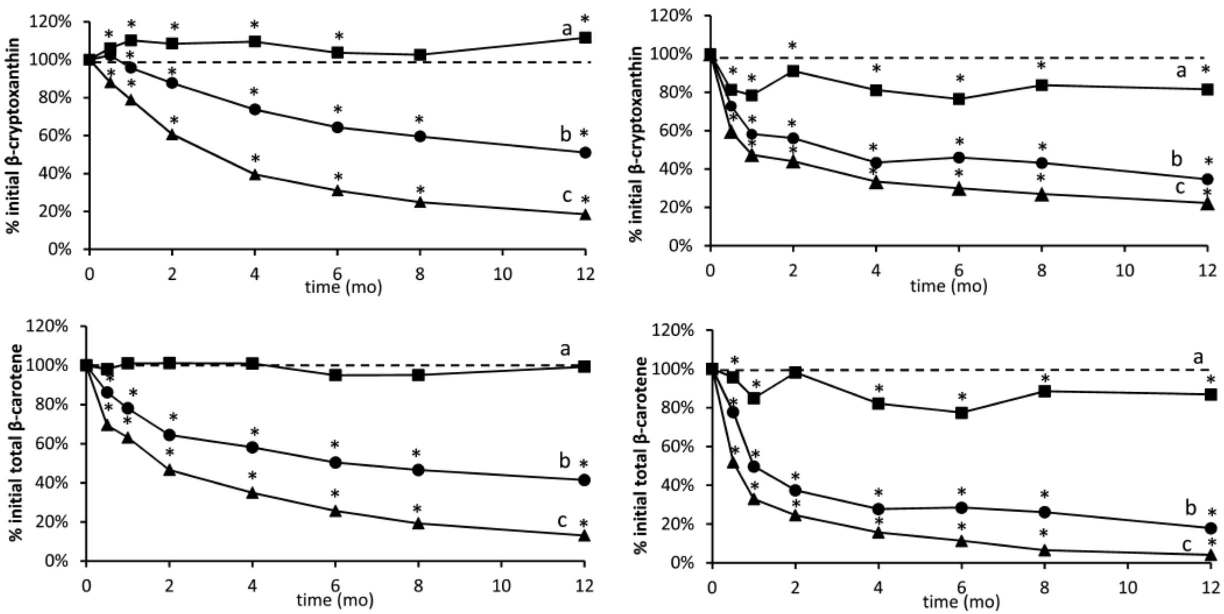
Apocarotenones

Lighter carbonyl compounds (i.e. norisoprenoids)

ACS OMEGA
Article
Cite This: ACS Omega 2017, 2, 7320-7328
http://pubs.acs.org/journal/acsodf

Retention of Carotenoids in Biofortified Maize Flour and β-Cryptoxanthin-Enhanced Eggs after Household Cooking

Margaret Sowa,[†] Jiaoying Yu,[†] Natalia Palacios-Rojas,[‡] Shellen R. Goltz,[†,∥] Julie A. Howe,[†,⊥] Christopher R. Davis,[†] Torbert Rocheford,[§,#] and Sherry A. Tanumihardjo*,[†]



- Degradation rate is higher at higher temperature
- BCX degradation rate is lower than BC degradation rate

Carotenoid retention is Higher in high Particle size flour

cooking method		lutein	zeaxanthin	β-cryptoxanthin	all-(E)-β-carotene
		carotenoid retention (%)[b]			
whole-grain (1.7 mm)					
	flour	100	100	100	100
	porridge	108 ± 7.2[a]	112 ± 4.6[a]	110. ± 2.1[ab]	125 ± 4.9[a]
	tortillas	82.7 ± 29.6[b]	80.7 ± 26.3[c]	93.1 ± 11.6[c]	92.8 ± 13.2[de]
	puffs	43.9 ± 12.9[c]	55.8 ± 11.5[d]	119 ± 11.8[a]	111 ± 8.7[b]
	muffins	98.5 ± 20.9[ab]	103 ± 25.5[ab]	106 ± 18.2[b]	108 ± 19.0[bc]
sifted flour (0.6 mm)					
	flour	100	100	100	100
	porridge	98.2 ± 11.0[ab]	99.8 ± 11.7[ab]	91.6 ± 4.2[c]	98.2 ± 5.4[cd]
	tortillas	99.0 ± 4.6[ab]	92.6 ± 3.17[bc]	91.7 ± 2.2[c]	86.7 ± 2.3[de]
	puffs	51.7 ± 7.3[c]	53.4 ± 7.0[d]	88.0 ± 6.5[c]	86.1 ± 8.3[de]
	muffins	98.1 ± 16.1[ab]	91.2 ± 13.1[bc]	89.9 ± 6.6[c]	82.8 ± 14.9[e]

Food Chemistry 232 (2017) 60–66

ELSEVIER

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Carotenoid retention in biofortified maize using different post-harvest storage and packaging methods

Víctor Taleon [a,*], Luke Mugode [b], Luisa Cabrera-Soto [c], Natalia Palacios-Rojas [c]

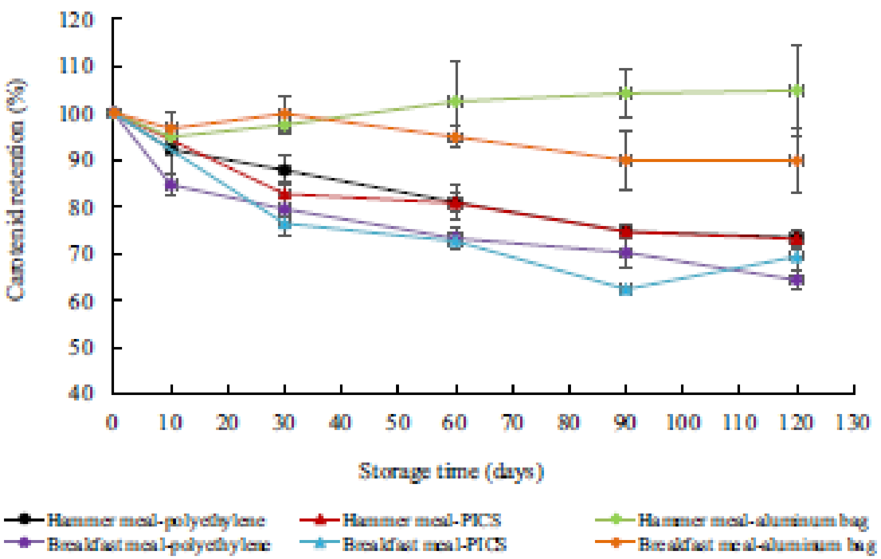
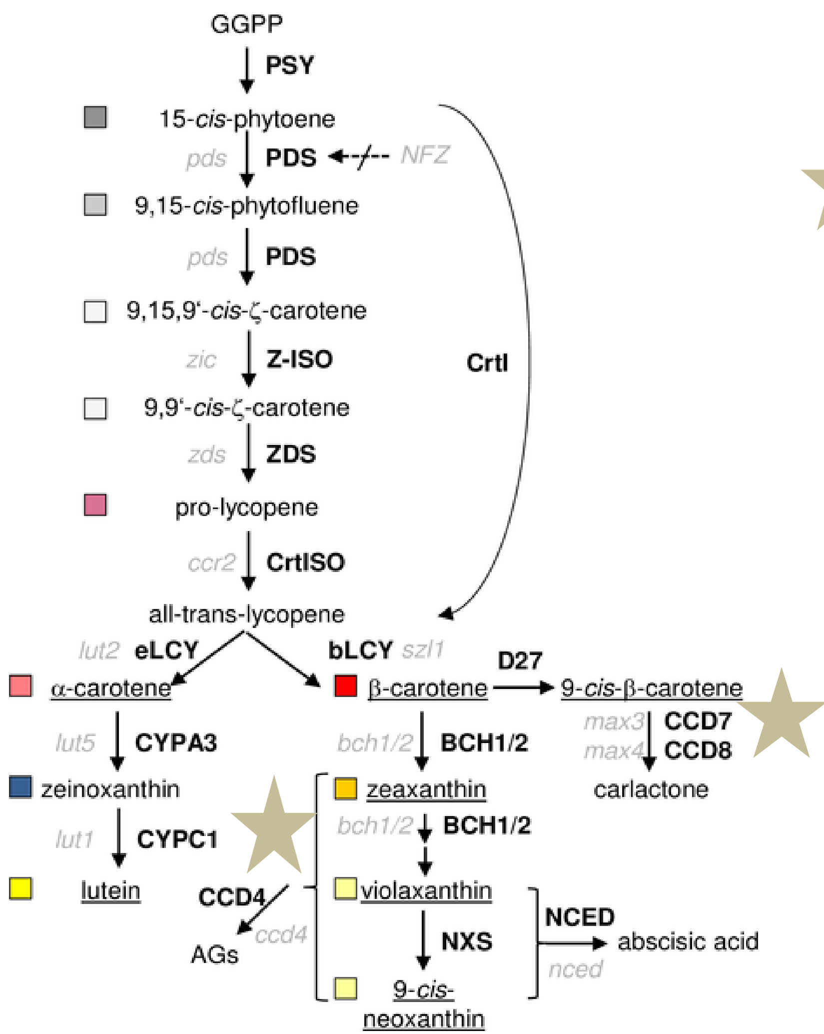


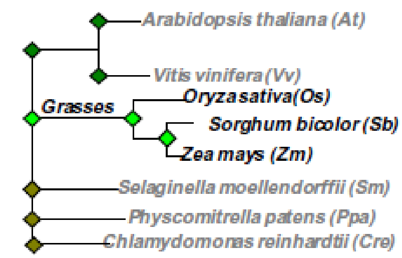
Fig. 2. Effect of storage method on pVAC retention in breakfast meal and hammer meal made from orange maize variety GV664A and stored using different packaging methods for 120 days under ambient conditions in Zambia.

- Degradation rate of βCX was 51% lower than βC during storage of orange maize grain.
- Grain storage methods with 16% oxygen level reduced carotenoid degradation by 9.1%.
- PVA more stable in hammer meal compared to breakfast meal (particle size)

Fig 1. Carotenoid synthesis and cleavage pathway in higher plants.



CCD1 and CCD4 cleavege into volatile apocarotenoids



(Vallabhaneni et

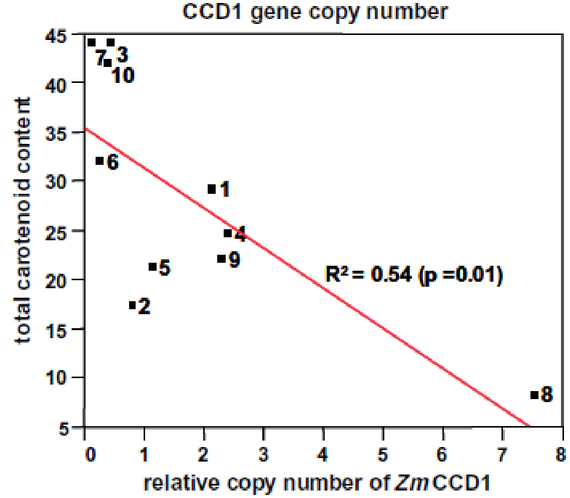
Schaub P, Rodriguez-Franco M, Cazzonelli CI, Álvarez D, Wüst F, et al. (2018) Establishment of an Arabidopsis callus system to study the interrelations of biosynthesis, degradation and accumulation of carotenoids. PLOS ONE 13(2): e0192158.
https://doi.org/10.1371/journal.pone.0192158
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0192158

PLOS | ONE

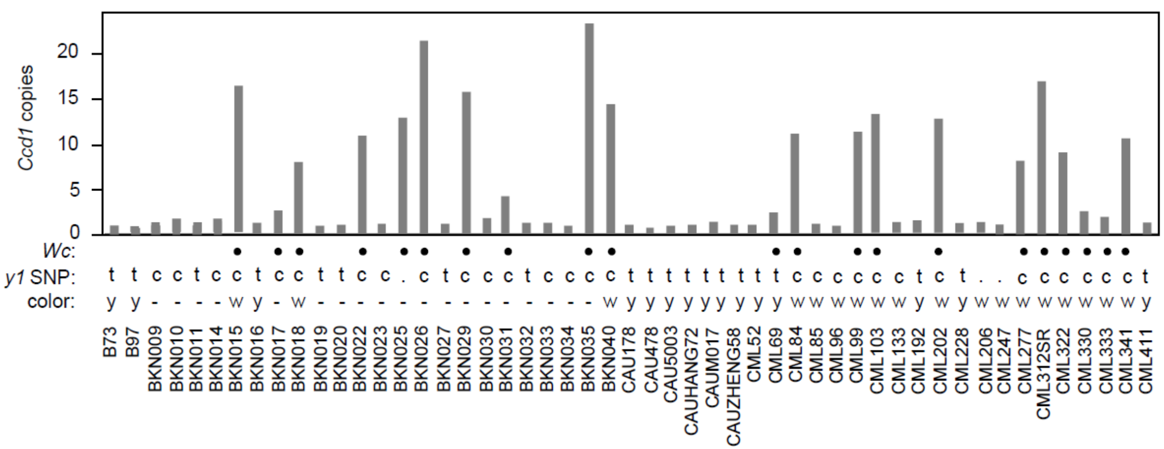
Structure and Origin of the *White Cap* Locus and Its Role in Evolution of Grain Color in Maize

Bao-Cai Tan, Jiahn-Chou Guan, Shou Ding, Shan Wu, Jonathan W. Saunders, Karen E. Koch and Donald R. McCarty

GENETICS *Early online February 3, 2017; https://doi.org/10.1534/genetics.116.198911*



ig. 6. Gene copy number of *ZmCCD1* relative to *ZmPDS*. Mean of three replicates standard deviation. Line indicates linear fit line. Inbred lines are: 1, A619; 2, B73; , B37; 4, CL7; 5, C131A; 6, DE3; 7, KUI2007; 8, NC300; 9, SD44; 10, TZI18.



No differences in CCD1 gene copy number in maize breeding lines with high and low carotenoid degradation rate

Perspectives

1. **All yellow maize breeding for SSA (~10% of total market for maize) can be potentially replaced with PVA yellow/orange maize hybrids.**
2. **Lowland tropical and mid-altitude/transition agro-ecology-adapted PVA hybrids (12-15 ppm and more) for deployment in selected countries with high per capita maize consumption**
3. **"Bridging" breeding program for PVA maize** with enhanced development and application of tools to efficiently introgress PVA trait into elite white germplasm, while not compromising on the yield gains.
4. Simultaneous and targeted efforts to bring **greater awareness among the white maize-consuming communities about nutritional benefits of PVA-enriched maize**, and to stimulate demand from consumers as well as seed companies.
5. **Better understanding of PVA degradation in maize and strategies to enhance PVA stability**

High-Zn Maize

High Zinc breeding scheme

Progenitores
(ACG, Producibilidad y Zn)

F1s

F2s
(100-150 autos)

DH

S2s
(10000-20000)

S4s-S5s

Ensayos Etapa I
(500-1000, 3 sitios en Mex)

Ensayos Etapa II
(100-200, 12 sitios)

Ensayos Etapa III
(50-100, 20 sitios)

Validación
(2-5, 25-30 sitios)
Regulación país

Análisis de Zn por XRF
(USD 2.6 por muestra)

Análisis de Zn por ICP
(USD 7.7 por muestra)

1950s- 1960's

1970s

1990s-2003

2003 onwards

2017

Mexican landrace, Colombian and Venezuelan landraces

(Tuxpeño) (Comun & Chococeño) (Puya & Cubano amarillo)

Improved germplasm

(Tuxpeño; ETO composite)

Quality protein maize

Populations 62 and 63

QPM CMLs

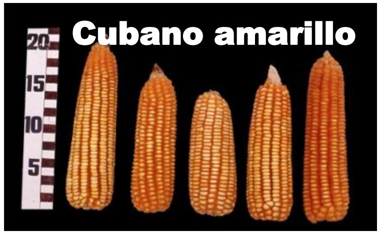
(CML176, CML491, CML492)

+ Lines derived from La Posta Sequia

High Zn maize

Hybrid	Zn (ppm)
CLTHWZN15010	34.9
CLTHWZN15011	36.1
CLTHWZN15013	33.1
CLTHWZN16051	37.7
CLTHWZN16102	29.8

Foto: Mayolo Leyva



Fotos CONABIO: http://www.biodiversidad.gob.mx/usos/maices/grupos/; Dra. Creucí Maria Caetano- UNAL, Colombia

High-Zn maize hybrids evaluated at 20 locations in Latin America under rainfed conditions

Hybrid Code	Grain Yield t/ha	Silking Date d	% Ear Rots %	% Corn Stunt %	Zn (µg/g) 3 sites Mex
CLTHW14003 (Reference Check)	5.95	59.2	6.8	6.9	26.7
CLTHWZN15010	5.46	59.8	7.8	7.5	34.9
CLTHWZN15011	5.28	58.8	9.9	10.9	36.1
CLTHWZN15013	5.25	60.7	17.4	7.2	33.1
CLTHWZN15017	5.21	59.3	6.8	7.3	34.7
CLTHWZN15004	5.14	59.6	12.6	6.9	32.7
CLTHWZN15018	5.12	61.6	13.3	8.4	27.3
CLTHWZN15005	5.02	59.6	15.9	6.2	30
CLTHWZN15012	4.99	58.5	8	10.9	33.7
Local Check 1	4.82	59.5	12.5	5.3	
CLTHWZN15015	4.74	61.4	12.3	7.1	27
Local Check 2	4.6	59.7	8.1	6.9	
Trial Mean	**5.13**	**59.82**	**10.94**	**7.63**	**31.6**
Heritability	**0.71**	**0.92**	**0.64**	**0**	
LSD	**0.53**	**0.75**	**5.96**	**4.98**	
CV	**5.13**	**0.63**	**27.24**	**32.62**	

- The best high Zn hybrid yielded about 9% lesser that the CIMMYT reference check and at least 1.0 t/ha better than the local check.
- Yield gap is significantly smaller for high Zn maize; however, the grain quality and color need to match better the market/consumer requirements.

HarvestPlus
Breeding Crops for Better Nutrition

Trait		Zn
Adj_Mean	L1	26.28
	L2	25.26
	L3	29.60
	Across	27.036
Range	L1	15.2-42.5
	L2	14.76-50.15
	L3	17.1-39.8
	Across	17.11-43.69
LSD	L1	5.11
	L2	4.62
	L3	6.30
	Across	5.41
CV	L1	9.91
	L2	9.34
	L3	10.87
	Across	10.22
h^2	L1	0.78
	L2	0.83
	L3	0.76
	Across	0.86
G		<2.2e-16***
G*E		<2.2e-16***

923 maize lines

892 Non- QPM

19% of the lines are high
In zinc > 30ppm

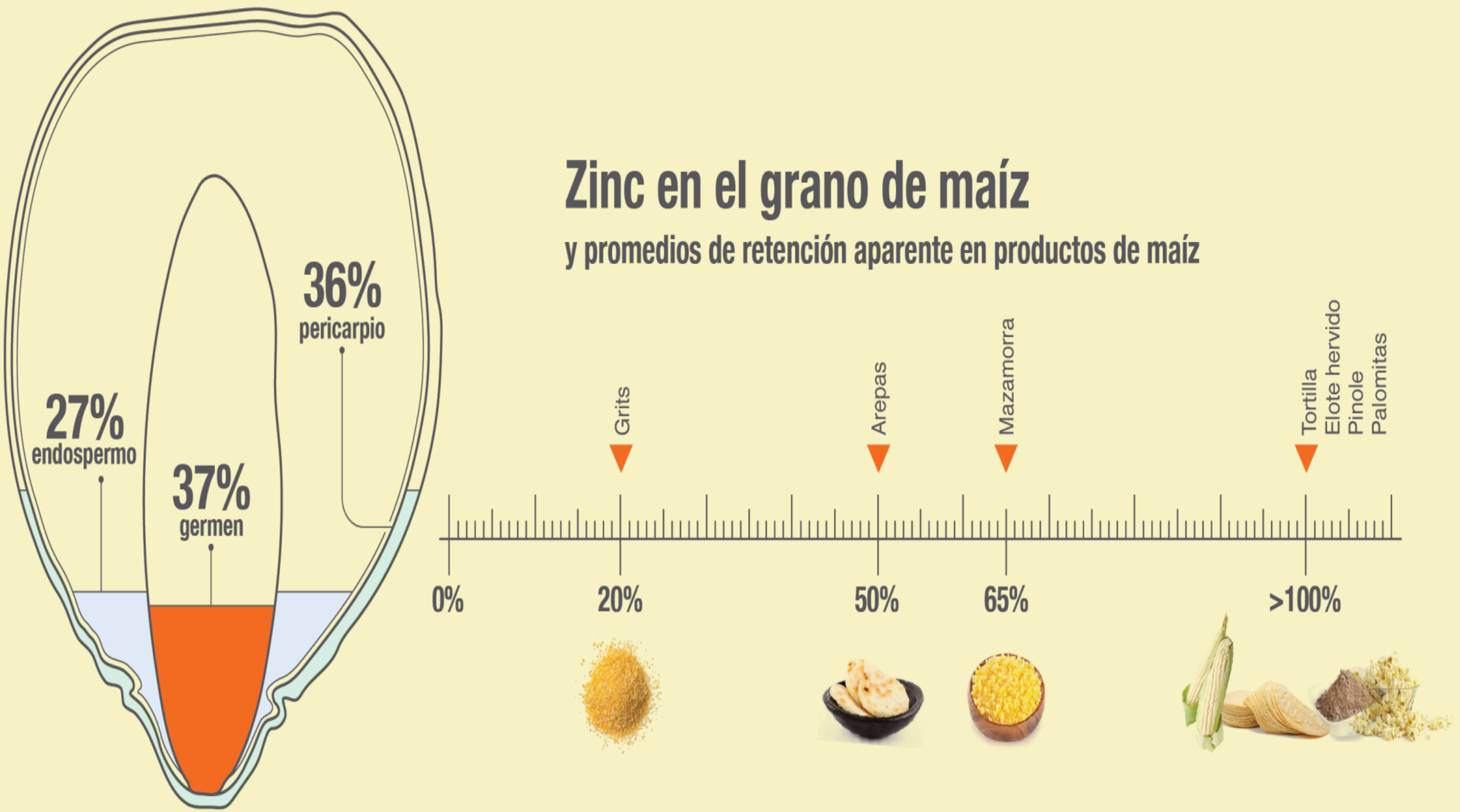
31 QPM

33% of the lines are hig
In zinc > 30ppm

Locations (L)	TL12A	CE12B	TL13A
TL12A	1	0.65***	0.68***
CE12B	0.62***	1	0.62***
TL13A	0.68***	0.54***	1

Great potential to develop high Zn maize alone or in combination with better protein quality

Vemuri et al., TAG 2018 in press



Zinc en el grano de maíz
y promedios de retención aparente en productos de maíz
36%
pericarpio
27%
endospermo
37%
germen
Grits
Arepas
Mazamorra
Tortilla
Elote hervido
Pinole
Palomitas
0%
20%
50%
65%
>100%

Validation of SNPs associated with kernel Zn content in maize biparental populations and breeding populations

SNP	GWAS p value	SF-QTL p value	SF-QTL R^2
S8_125472630	3.95E-05	0.0181	7.36
S9_151265550	5.50E-05	1.68E-03	6.55
S7_173181689	6.74E-05	5.21E-09	28.38
S7_8305436	9.37E-05	0.0051	5.20
S10_60224874	8.18E-04	0.00923	9.26

Trait	LG	Position	LeftMarker	RightMarker	LOD	PVE(%)	Add	LeftCI	RightCI	GWAS_SNP in QTL interval
DTPYC9-F13-2-1-1-2-B-B/CML312: Celaya										
Zn	7	5	S7_173837564	S7_173448589	3.2158	14.3927	-1.5016	0	8.5	S7_173445764
CML 465/CML451:Celaya										
Zn	8	109	S7_171537807	S7_173277939	9.071	22.5383	-2.2921	106.5	111	S7_173181689
Zn	11	34	S9_152192799	S9_151306650	3.932	8.7968	1.4499	27.5	37.5	S9_151265550
DTPYC9-F13-2-1-1-2-B-B/CML312: Celaya and Tlaltizapan										
Zn	1	27	S1_272041829	S1_252374951	3.9659	14.7537	-0.9899	18.5	31.5	S1_254988179
Zn	9	54	S9_129767650	S9_138240205	4.567	11.8412	-0.8407	46.5	56.5	S9_136390177; S9_136389202
CML503/CLWN201: Celaya and Tlaltizapan										
Zn	1	43	S1_273696896	S1_246409151	3.3941	8.3573	-1.1587	35.5	52.5	S1_254988179
CML 465/CML451:Celaya and Tlaltizapan										
Zn	8	108	S7_171537807	S7_173277939	10.8134	18.2844	-1.5537	106.5	111	S7_173181689
Zn	11	34	S9_152192799	S9_151306650	4.0385	6.6929	0.8344	27.5	37.5	S9_151265550

HarvestPlus
Breeding Crops for Better Nutrition

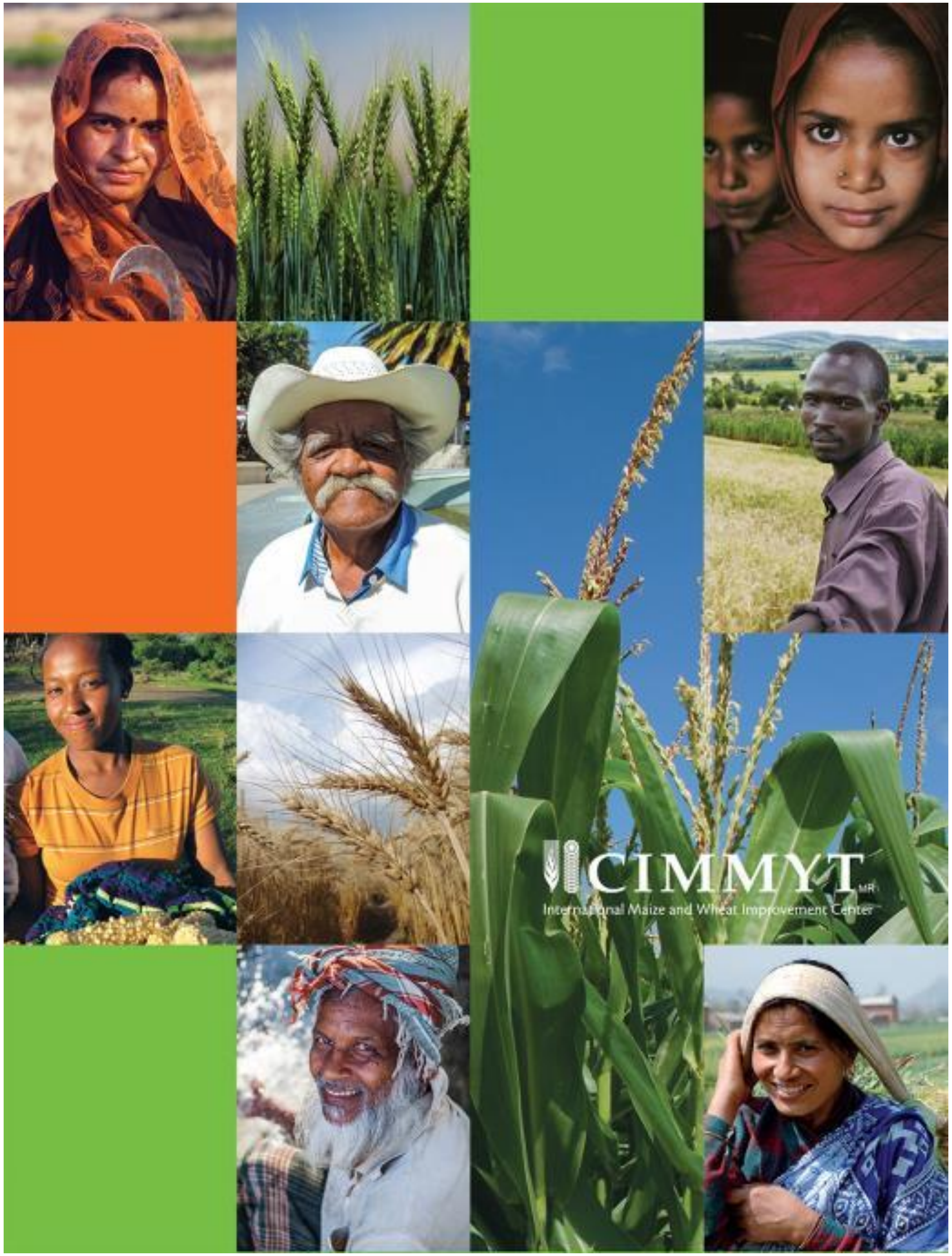
High-Zn Maize (at least 30-35 ppm)

1. Potential Target Countries (for further expansion in a phased manner)

2. High-Zn breeding program:

- Identifying high-Zn trait donors in non-QPM germplasm
- Developing a suitable phenotyping/testing network
- Validating markers for high-Zn in elite lines and developing a strategy for marker deployment
- Introgressing high-Zn trait into elite white germplasm for targeted agro-ecologies in SSA and LA
- Bringing greater awareness among the white maize-consuming communities about nutritional benefits of high-Zn

3. Zinc efficacy studies in Latin-American populations



Thank you for your interest!

HarvestPlus
Better Crops • Better Nutrition

MAIZE