



#### A novel breeding approach to develop faster cooking biofortified common beans (Phaseolus vulgaris L.) for East Africa

#### Clare Mukankusi Mugisha August 2023

The Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT) is part of CGIAR, a global research partnership for a food-secure future

#### Outline



#### Introduction

#### Project justification

#### Breeding approach

Founder 1

Founder 2

#### Current status



#### Alliance-Bean Program: What we do



# Delivering better bean varieties for income, nutrition, and resilience

With our partners, we develop improved bean <sup>10 country</sup> varieties that show resilience under harsh growing conditions resulting from climate change



#### **Common bean (***Phaseolus vulgaris* L.**)**

- **Important staple:** >400M consumers
  - East Africa highest production and per capita consumption
- Income crop : Rapid evolution from traditional subsistence to a market-oriented crop
  - >USD 500M in sales
- Nutrition and health: Source of protein, micronutrients iron (Fe) and Zinc (Zn), vitamins, dietary fibre, and low GI
- Soil fertility: Legumes ability to fix nitrogen in the soil





#### **Constraints to bean business in East Africa**

- Diverse market classes
- Diverse constraints
- Diverse research priorities
- Limited resources
- Unequal capacity





#### ABIOTIC

- Drought (>60% area)
- Heat (beans not viable in 50% area by 2050)
- Low soil fertility (N deficiency; 60% Africa, P deficiency; 50%, AL toxicity 40%)
- Excessive rainfall
- Cold

#### • BIOTIC:

- Pests (field and post harvest)
- Diseases (fungal, bacterial and viral)
- Other factors:
  - Population pressure migration to drier areas, less fertile
  - Bean utilisation and marketability



FIGURE 12. Estimated mean yield loss (kg/ha) by AFBE, due to 15 constraints identified as very important to bean production in Africa, assuming an average total loss to all constraints of 1000 hg/ha.





#### What led to the development of this project?

## Long cooking time in beans impacts women and children

- Lost opportunities (school, work)
- Deforestation risk to environment
- Collecting firewood = great personal risk
- Smoke inhalation = health risk
- Lost nutrition



#### Iron and zinc deficiency impacts women and children

WHO concerns:

- Anaemia
- Wasting, stunting and underweight
- Reduced cognitive ability
- Reduced immune function





## Common bean a major staple in East and Central Africa is an accessible avenue of increasing Fe and Zn consumption





# Customer analysis along the bean value chains: Example of Uganda (Katungi *et al,* in press)

Customer	Desired traits	Market size (#people)	Growth Potential	Sex	Geographical location
<b>1</b> .Middle/High income & consumers	Shorter cooking time Sweet taste, softness	3,053,250	moderate	ø	peri-urban, urban
<b>2</b> . Low-income household consumers	<b>Shorter cooking time</b> , Soup thickness, Larger grain sizes, good taste, Softness , <b>Swelling grains</b>	13,242,300	High	ø	rural
<b>3</b> .Low end Restaurants and fast foods	<b>Shorter cooking time</b> , Larger grain size, Soup thickness, Sweet taste, softness, <b>Swelling</b> grains	11,062,500	High	Ŷ	Urban/Peri-urban
<b>4</b> .Lower Income schools	<b>Shorter cooking time</b> , Larger grain size, Soup thickness, <b>Swelling grains</b> , Soup color, <b>Resistant to storage pest attack</b> , Softness	7,500,000	High	8	-Urban/peri-urban
<b>5</b> .Prisons	Shorter cooking time, Resistant to storage pest attack, Softness	55,200	Low	δ	Urban/rural

# Our solution: Breed rapid cooking beans, biofortified with Fe and Zn

#### (with high yield and environmental resilience)

#### ACIAR project CROP-2018-132: Rapid cooking beans

















Australian Centre for International Agricultural Research





	Target Product Profile					
larket Segment Description	Medium and large seeded bush beans	s for East Africa				
	Reans FSA FAF Medium and large so	eed Red/red mottled & sunar	Low & Mid altitude Rush Rainfed Farly			
ron	Beans					
ne CGIAR Region	Fast Africa Southern Africa					
ne CGIAR Region	Fast Africa					
ountries	Burundi: 201 300: Ethionia: 144 780: Ker	va. 1 004 087: Rwanda: 192 281: 1	Canzania: 996 በበቡ Huanda: 600 በበቡ DRC: 385 611			
lectares in ONE CGIAR sub	3.524.059	iya. 1,00 1,007, Nwanaa. 102,201, 1				
egion						
laterial Type	Variety					
iological Region/Eco System	Low and mid altitude areas					
rowing season	Burundi: (March-July); Ethiopia: (June-( (February-May and September-Decemb	October); Kenya: (March-May); Rw er)	/anda: (September -December and March-May); Tanzania: (March-May) Uganda: (March-May	/ and Septembe	r-December); DF	RC:
	Trait	Scale	Min Score	Trait requirement	Improve trait	Threshol trait
olor	Red/Red mottled/sugar /yellow/kablanketi/white/brown	Varied, however red mottled commands 30% of current market share followed by sugars, reds and lastly the yellows	Varied, the types all require the same traits and farmers do switch among them based on the strength of a trait in any one of them e.g., taste, cooking time, yield etc.	Essential		Y
rocessing traits	Color retention (Color retention after canning/ precooking process)	Color before and after canning/ precooking process the same	>=local check (e.g CAL96)	Nice to have		
onsumption traits	Fast cooking (reduction in cooking time) for dry grain	cooking time in min	30% < commecial checks in ECA in specific market group	Essential	Ŷ	
lutritional Enhancement	Iron (Fe) grain content	mg per Kg	20-30%> commercial checks in specific grain classes	Essential	Y	
raits	Zinc (Zn) grain content	mg per Kg	10%> commercial checks in specific grain classes	Essential	Y	
ield	Gain yield (t/ha)	tons/ha	10%> commercial check (850-1500kg/ha); in specific grain class	Essential		Y
gronomic traits	Early maturity	Days afer planting (dap)	= < 75 dap ( RWR2245)	Essentail		Y
isease traits	Angular leaf spot resistance	1 to 9	<4 on the Disease severity scoring scale (presence of Phg2 gene); Bench marks: RWR22245, RWR2154, NAROBEAN3/MOORE8802, KATB1, JESCA, SAB713, Nyota)	Essentail		Y
nsect traits	Bruchid resistance	Presence of arcelin gene	Presence of arcelin gene	Nice to have		
ey Competitive Products			NABE 14; Selian 13; KAT B1; CODLMBOO3; Nyota			

#### Objective

• Goal to reduce cooking time by 30% and increase Fe grain content by 15% and Zn 10% after 5 cycles of annual recurrent selection

Based on diverse "founder" population (African bean panel)

Use pedigree and genomic information to generate accurate breeding values in a selection index, together with optimal contribution selection (OCS) to accelerate breeding in annual cycles

"Road-test" and release new varieties rapidly through PABRA system









#### The Team







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#### **Genetics of Cooking Time**

- Large genetic diversity in cooking time
  - 20-120 mins in a Mattson cooker
  - 1.5-3.5 hrs traditional cooking methods
- Relatively low G x E variance
- High heritability (*h*<sup>2</sup> = 0.74, Jacinto-Hernandez et al. 2003)
- Genomic regions on three chromosomes (Cichy et al. 2015)
- Relatively few QTL (Berry et al, 2020; Diaz et al, 2021)



#### **Iron and Zinc Grain Content**

- Iron and zinc content are quantitatively inherited and positively correlated (Blair *et al., 2009*)
- Influenced by the environment, but varies depending on the source genotype (Cichy *et al.*, 2009)
- Shorter cooking time correlated with improved Fe bioavailability (Wiesinger et al. 2018).



#### BRÍO Breeding: from canola to beans <u>https://research.aciar.gov.au/rapidcookingbeans</u> <u>/brio</u>

#### **BRÍO Breeding**

- con brío: with vigour, vitality, energy, strength
- **B** Breeding values (BVs) accurately predicted from analysis of phenotypic and genomic and/or pedigree relationship data accumulated over cycles
- **R** Rapid cycles of recurrent selection (2-3 years)
- Í Index selection on BVs weighted for economic value
- **O** Optimized mating designs for sustainable and superior genetic gain





**Breeding Scheme:** 12 month cycles for crossing, seed increase, and selection for CKT, Fe, Zn in the lab, followed by field trials by partners

#### LEGEND

Linked to BMS

- → Seed flow
- 🗥 Data flow to BMS
- > Data exported from BMS for data analysis
- Results of data analysis
- ···> MateSel output
  - Oecision making process at project coordinating team
- Decision pathway
- X Decision making process at project partners

🗂 Crossing list



CGIAR

#### Summary: Population development and advancement

Population	Number candidates/tr ials	Training population	Number of markers	Cycle 1	Cycle 2	Market groups
Founder 1 Cycle 1	4 trials, 258	161 entries	Two SNP panels	67 parents (160 crosses)	206 crosses; 900+ progeny to be field tested	Six
Founder 2 Cycle 1	33 trials, 876/358 genotypes	141 entries	Two SNP panels	120 crosses		Four and emphasis on bc3
Cycle 2 begins			Mid density (SNP platform)	n/a	88 crosses	Four



## Founder 1 population

#### **Parental selection**

- African diversity panel
- Two trials: 258 lines (key grain classes)
  - Grain yield (GY)
  - Cooking Time (CKT): 50 g of dry seed ground into a powder; Seed Fe and Seed Zn concentration determined using EDXRF spectrometry
  - SeedFe and SeedZn: 25 seeds randomly picked from clean harvested seed from each plot
- Two SNP panels:
  - Panel 1: 770 SNP array with 94 genotypes was a
  - Panel 2: More than 20,000 SNPs with 298 genotypes based on DArT



#### **Towards an Optimised mating design**



Alliance Bioversity & CIAT

#### Genomic Breeding Values and Heritability in the African Bean Panel

- Imputation of a genomic relationship matrix for 161 genotypes from two SNP panels
- GEBVs for GY, CKT, SeedFe and SeedZn calculated



Dr Li Li, University of New England-Armidale

Trial/site	Trait	No. candidates with GEBV values	Mean value of candidates in trial	GEBV minimum	GEBV maximum	Genomic heritability (h <sup>2</sup> ) (* P<0.05; ** P<0.01, NS not significant)
Bush2015	Yield (kg/ha)	161	986	-449	+570	0.40 *
Bush2017	Cooking time (min)	161	77.6	-27.0	+59.0	0.81 **
Bush2015	Fe (ppm)	161	64.9	-8.4	+10.5	0.53 *
Bush2015	Zn (ppm)	161	30.4	-1.6	+1.8	0.28 NS



#### **Correlation of genomic estimated breeding values for GY, CKT, Fe and Zn**

	GY	CKT	Fe
GY			
CKT	-0.25 (0.10)		
Fe	0.13 (0.09)	-0.23 (0.10)	
Zn	0.15 (0.09)	-0.22 (0.10)	0.64 (0.05)



#### **Optimized selection index-Optimized economic weights**

- Economic weights (EW) on GEBV for GY, CKT, Fe and Zn reflecting current market prices in east Africa (0.85 US\$/kg) and average GY in the trials (869 kg ha<sup>-1</sup>)
  - 15% price bonus for 10% shorter cooking time and a 10% price bonus for 10% improvement in Fe and 5% price bonus for 10% Zn content.
- EW optimized as implied-EW's using the tactical desired gains approach in the program DESIRE



#### **Assigning Economic Indices to Genotypes**

- Economic index of an individual genotype is calculated by adding the value (US\$/ha) of its GEBVs for each trait, that is, (a) + (b) + (c) + (d)
- It follows that a genotype with

(a) GEBV GY +0.1 t/ha
(b) + GEBV CKT -1 min
(c) + GEBV Fe +1 ppm
(d) + GEBV Zn +1 ppm

= economic index US\$132.95 /ha

(relative to population mean \$0)

GEBV GY (%)			-10%	0%	+10%
GEBV GY (kg/ha)			-100	0	+100
GEBV GY (\$/ha)		(a)	-\$85.00	\$0.00	+\$85.00
GY (kg/ha)			900	1000	1100
			Contri	bution to index	(\$/ha)
GEBV CKT (\$/ha)	-1 min	(b)	+\$14.71	+\$16.35	\$17.98
GEBV_CKT (\$/ha)	0 min	(b)	\$0.00	\$0.00	\$0.00
GEBV_CKT (\$/ha)	+1 min	(b)	-\$14.71	-\$16.35	-\$17.98
GEBV_Fe (\$/ha)	+1 ppm	(c)	+\$11.77	+\$13.08	+\$14.38
GEBV_Fe (\$/ha)	0 ppm	(c)	\$0.00	\$0.00	\$0.00
GEBV_Fe (\$/ha)	-1 ppm	(c)	-\$11.77	-\$13.08	-\$14.38
GEBV_Zn (\$/ha)	+1 ppm	(d)	+\$12.75	+\$14.17	+\$15.58
GEBV_Zn (\$/ha)	0 ppm	(d)	\$0.00	\$0.00	\$0.00
GEBV_Zn (\$/ha)	-1 ppm	(d)	-\$12.75	-\$14.17	-\$15.58

#### **Optimized selection index**

- Inputs into DESIRE; EW's, h2 for each trait, SD of BVs, and the genetic and phenotypic correlations between traits.
  - 1º emphasis was to reduce CKT
  - 2° emphasis was to increase Fe and Zn while also improving GY
- Optimized selection index (*GBLUPindex<sub>i</sub>*) calculated for each individual *i* from *j* traits (Cowling *et al.*, 2017)

• GBLUP index<sub>i</sub> = 
$$\sum_{j=1}^{nTraits} e_j \cdot GEBV_{i,j}$$



## **Generation of Optimized Mating Design**

- An optimised mating design was generated with OCS in 'Matesel' (Kinghorn and Kinghorn 2019)
- 'Matesel' provides a balance between next-generation genetic gains and parental coancestry
  - **"MateSel** is a tool that enables breeders to optimize breeding outcomes by creating a suggested mating list based on a group of candidate sires and dams"
- A conservative approach to OCS was used to favour retention of genetic diversity while advancing the population mean index

Included consideration of consumer preferred grain market classes.

- red mottled beans-Uganda, Kenya, Burundi and Rwanda
- yellow beans in Tanzania, Uganda, Rwanda and Burundi
- white beans in Ethiopia and Madagascar;
- Sugar bean (cranberry) in Tanzania, Malawi, Zimbabwe and Uganda



#### Optimized Mating Design in 6 Market Groups (Founder 1)

Market Class Group	Seed color	Candidates with phenotypes	Candidates with GEBVs	Candidates selected for mating	Times used in mating
Gp1	Cream/Beige/Yellow	51	28	13	24
Gp2	White	43	18	8	18
Gp3	Red	61	42	14	46
Gp4	Black/Purple/Calima	62	30	13	34
Gp5	Striped/Pinto	42	30	14	28
Gp6	Speckled/Cranberry/Other	19	13	5	10
Total		278	161	67	160





#### **Predicted Outcome from Founder 1 crossing**

- A major improvement in population mean for the Economic index : +286.77 US\$/ha
- 6.2% increase in GY
- 7.3% reduction in CKT
- 1.1% Increase in SeedFe
- 0.7% increase in SeedZn
- Achieved increase in population co-ancestry of 0.0753

Trait	Predicted change in trait in progeny	Predicted change in trait in progeny (%)
Economic index (US\$)	+ 286.77 US\$/ha	
Yield	+ 61 kg/ha	+ 6.2%
Cooking time	- 6.0 min	- 7.3%
Fe	+ 0.7 ppm	+ 1.1%
Zn	+ 0.2 ppm	+ 0.7%



#### Founder 1 Cycle 1 (2019)





#### **Correlation of genomic estimated breeding** values - Founder 1 cycle 1

CKT increases as Fe/Zn increases

CKT decreases as WAC increases

#### **Zn goes up as Fe increases**

iron



...

••

0.98	CKT12		Small	seeds ha	ave high i
0.42	0.48	Fe			
0.26	0.27	0.58	Zn		
-0.11	-0.12	-0.27	-0.12	DryWt	
-0.79	-0.81	-0.29	-0.26	0.18	WAC
	0.98 0.42 0.26 -0.11 -0.79	0.98CKT120.420.480.260.27-0.11-0.12-0.79-0.81	0.98CKT120.420.48Fe0.260.270.58-0.11-0.12-0.27-0.79-0.81-0.29	0.98CKT12Small0.420.48Fe0.260.270.58Zn-0.11-0.12-0.27-0.12-0.79-0.81-0.29-0.26	0.98       CKT12       Small seeds had         0.42       0.48       Fe         0.26       0.27       0.588       Zn         -0.111       -0.122       -0.277       -0.122       DryWt         -0.799       -0.811       -0.299       -0.266       0.18

**CKT20** 



### Founder1 Cycle2 crossing

## **BRÍO in action**

### **BRIO - Rapid cycles of early-generation selection**





#### S<sub>0</sub> progeny segregating for CKT



Time since start of cooking (min)



# Multivariate genomic analysis of laboratory traits measured on Founder1 Cycle1 $S_{0,2}$ and $F_{2,4}$ progeny

CKT20	= time from start of cooking to 20 <sup>th</sup> pin drop (min)
CKT12	= time from start of cooking to 12 <sup>th</sup> pin drop (min)
SeedFe	= Fe content in dry bean powder after cooking (ppm)
SeedZn	= Zn content in dry bean powder after cooking (ppm)
DryWt	= weight of 25 beans (g)
WAC	= % increase in weight of 25 beans after 18 hours soaking

Mid density SNP panel of approx 1800 markers

## Combined pedigree and genomic relationship matrices = H-BLUP analysis



#### BCMNV resistance important in East Africa

Α

С

SNP marker (bc-3a) for bc3 resistance gene

Resistant SNP allele:

Susceptible SNP allele:

Presence of resistant allele in Founder1 S<sub>0</sub> & F<sub>2</sub> progeny Homozygous resistant (AA): 26

Heterozygous (AC): 82

Homozygous susceptible (CC): 800





### Devote one crossing group for bc3 resistance allele in MateSel

- Make 60 crosses where all progeny must be at least heterozygous for resistant SNP allele
- *also*, select for lower CKT and higher Fe and Zn in these crosses
- allows future genetic gain for CKT, Fe, Zn and BCMNV resistance in this group



## Generate optimised crossing designs for 3 groups

- **Group 1 (94 crosses):** primary goal to reduce CKT, and secondary goal to increase Fe and Zn
- Group 2 (46 crosses): primary goal to increase Fe and Zn, and secondary goal to reduce CKT
- **Group 3 (66 crosses):** primary goal to retain BCMNV resistance, and secondary goals to reduce CKT and increase Fe and Zn
- Total = 206 crosses



# Predicted genetic gain in Founder 1 Cycle 2 progeny in MateSel





#### Year 3 Crossing Program - Founder 1 Cycle 2 (2021-22)



#### Cycle 1 breeding values – HBLUP analysis





## Heritability (h<sup>2</sup>)

	A BLUP	G BLUP	H BLUP
CKT20	0.04		0.17
Fe	0.13	NC	0.38
Zn	0.17		0.25

Combining pedigree and genomic relationship data improved heritability of all traits



### CKT Heritability (h<sup>2</sup>)

					H BLUP	HBLUP		
						(CKT12)		
	A BLUP	G BLUP	H BLUP	CKT20	0.19	0.34		
CKT20	0.04		0.17	CKT12		0.55		
Fe	0.13	NC	0.38	Fe	0.36	0.37		
7n	0.17				0.25	Zn	0.24	0.27
<b>۲</b> ۱۱	0.17		0.25	DryWt	0.33	0.33		
				WAC	0.25	0.25		

Including CKT12 in the analysis improved heritability of CKT20



#### Founder 2 population

Why?

Better data = better predictions = better parents = faster genetic gain

### Founder 2 crossing (2020)





#### OCS-predicted genetic gain in Founder 2 cycle 1 progeny



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The Plant Genome 📖 0

#### SPECIAL ISSUE: ADVANCES IN GENOMIC SELECTION AND APPLICATION OF MACHINE LEARNING IN GENOMIC PREDICTION

Multivariate genomic analysis and optimal contributions selection predicts high genetic gains in cooking time, iron, zinc, and grain yield in common beans in East Africa

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#### **RCBP Founder Crossing**



#### RCBP bean breeding 2019 - 2023

Year	2019		2020			20	021				20	022		2023
Month	J F M A M J J A S O	N D J F M	A M J J	A S O	N D J F M	A M J	J	A S O N	1 D J	F M	A M J	J A S O	ND.	F M A
Founder 1 Cycle 1	Crossing	F1 x F1	Genotyping	Seed in	oution	Partner field trials								
					CKT, Fe, 2	Zn								
Founder 2 Cycle 1					Crossing	F1xF1	L	Selfing	See	ed incre listribu	ease & ition	Partne	r field tria	als
									Ge	notypi	ng		СКТ	
Founder 1 Cycle 2										F0 (0	ounder1 ( CKT, Fe, Zr	Cycle 2 Crossi n, DryWt, WA	ng .C) Ge	enotyping
Cycle 2														Crossing

- > One crossing program every year at AB-CIAT, Kawanda
- $\succ$  S<sub>0</sub> and F<sub>2</sub> progeny evaluated at 6 PCs every year
- Accumulate genotypic and phenotypic data over years



#### Add grain yield data from partners - cycle 1 progeny field trials, test GY, seed colour





#### Combining Founder crosses- Fdr1 & Fdr2



#### Genetic correlations between sites- Grain yield



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#### Correlation of GEBVs among traits – Founder1 & 2

h2	GY	CKT goes up when GY increases 🥵											
GY	0.09	СКТ20	Fe and Zn high in small seeded beans										
СКТ20	0.31	0.21	Fe	Fe Iron and zinc go up together									
Fe	0.02	-0.01	0.35	Zn									
Zn	0.08	0.15	0.62	0.46	DryWt								
DryWt	-0.37	0.06	-0.30	-0.25	0.66	WAC							
WAC	-0.39	-0.16	0.15	-0.22	-0.05	0.17		.10					



# Genetic correlations between traits have major impacts on predicted genetic gain in all traits!!

#### **Predictions from OCS in MateSel show that:**

- ➢ GY decreases when CKT is given high weight
- ➢ Fe and Zn decrease when CKT is given high weight
- Seed DryWt decreases when Fe, Zn, GY are given high weights

# A compromise is needed! MateSel allows the user to manage predicted progeny distributions:

- > Minimise loss in seed DryWt in the population
- Achieve desired gains in Fe, Zn and GY while reducing CKT to a reasonable level

#### MateSel output: Founder 1 & Founder 2 cycle 2





## Summary

#### Cycle 1 & 2 progeny show strong predicted genetic gain

- $\succ$  CKT reduces by 9 % (cycle 1) and 10 % in cycle 2
- $\succ$  Fe increases by 4 % (cycle 1) and 6 % in cycle 2
- > Zn increases by 3 % (cycle 1 & 2)

#### What we don't know

- > Rate of genetic gain over cycles (next year)
- > % genetic gain compared with benchmark varieties (next year)



## Future goals

- Annual recurrent selection cycles
- Accumulate genotypic, pedigree and phenotypic data over cycles
- > Combined analysis every year (with help from UNE)
- Target seed size for each market?
- Include seed colour in decisions of crossing parent



### Advancement towards variety release

# Selection groups in ACIAR/CROP/132 (rapid cooking bean project, RCBP)



Cycle	1	2	3	4	5
%reduction in cooking time	5-10	10-15	15-20	20-25	25-30
Years in advancement and testing system and reach farmer	6	6	6	6	6
Actual year varieties reach the farmer	2025	2027	2029	2031	2033



#### Field testing with partners



#### Training within the project

- BMS: Field book download and upload
- Single site single trait with spatial designs
  - Identifying errors in data and optimising spatial model
- Multi-site field data and multi-year lab data BLUEs analysis
- NRM, GRM, H-matrix (ABLUP, GBLUP, HBLUP)
- Multi-trait analysis, Index calculation (Desire)
- Optimal Contribution Selection: OCS (MateSel)



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Australian Centre for International Agricultural Research





Alliance

























## Leveraging Genetic Innovation for Resilient African Food Systems in the wake of Global Shocks

#APBAConf2023



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# Thanks!