The "Sormaize" innovation journey: From concept to market

Alexander Bombom
Uganda

BOMVITAE AGRO INDUSTRIES LIMITED
Empowering communities . Improving livelihoods

NARO
National Agricultural Research Organisation

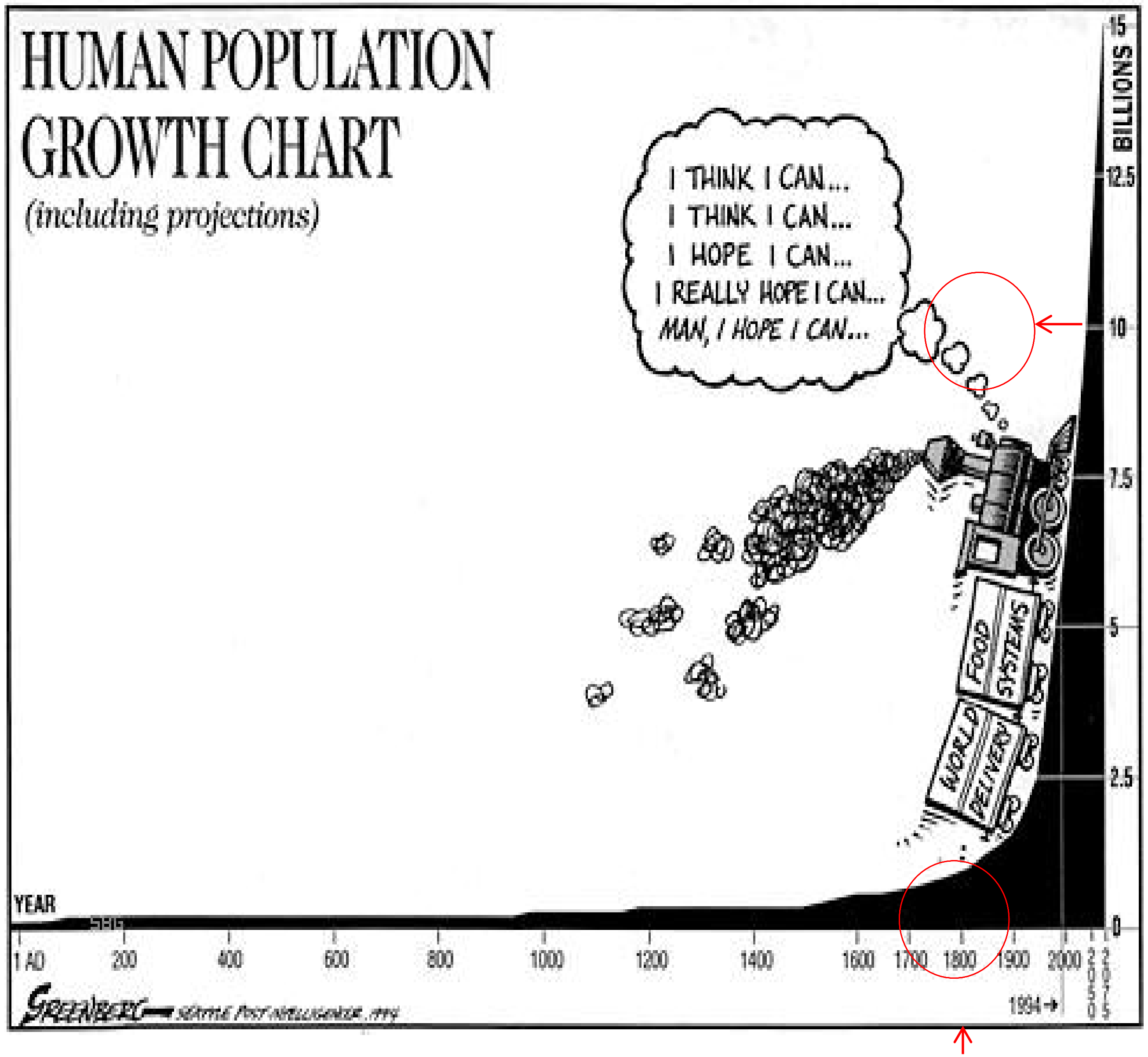
Outline

- Introduction – idea
- Wide crosses – what examples are there?
- “Sormaize”
- Applications in agricultural development
- Market Opportunities

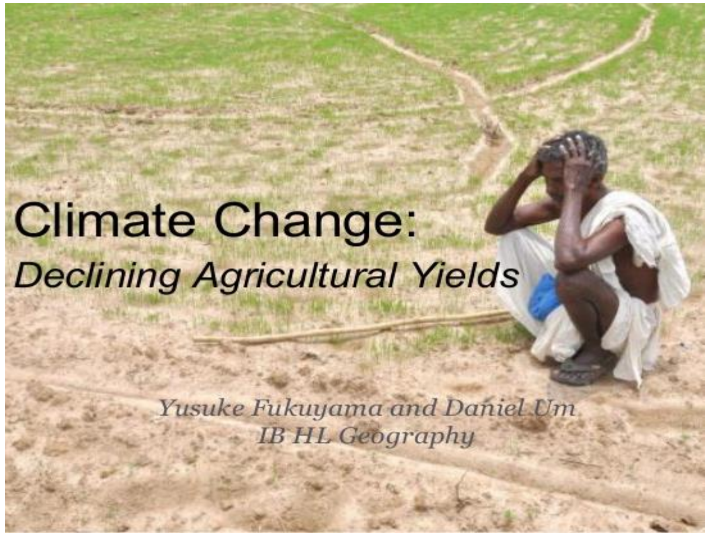
Introduction

Can we sustain the available finite resources??

- Food
- Feed
- Energy



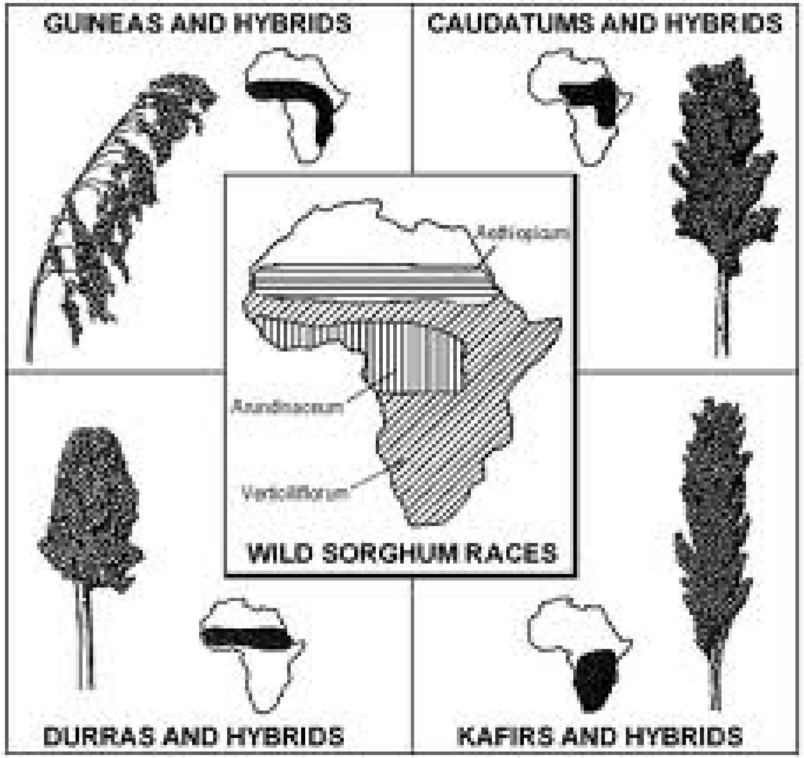
How do we cope with challenges?



- Intensified agriculture
- Climate smart and good agronomic practices
- Plant genetic resources
  - Pre-requisite for crop improvement
  - Pilar for food, feed, energy security globally

Crop biodiversity - Do we have what we need?

Sorghum



Maize

- Sources of genetic variation
    - Nature
    - Man made – genetic manipulation (conventional or biotech)
        - Interspecific and intergeneric crosses

Wide crosses

- Interspecific hybridization - species of the same genus
- Intergeneric hybridization - different genera of the same family.
- **Why wide crosses?**
  - Curiosity
  - Gene transfer
  - Novel genetic variation including new character traits absent in parents (increase crop diversity)
  - Haploid induction and doubled haploids
  - Novel crop species

Do we have examples? - Wide crosses in animals

Lion – 2n=38

X

Tiger – 2n=38

Tigon

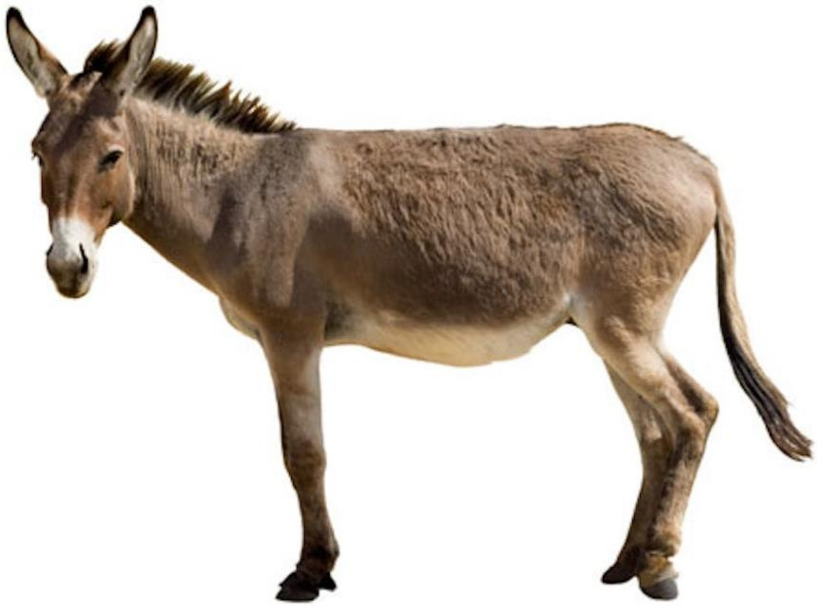
Tigon – 2n=38

Liger

Liger – 2n=38

Horse - 64 chromosomes

X

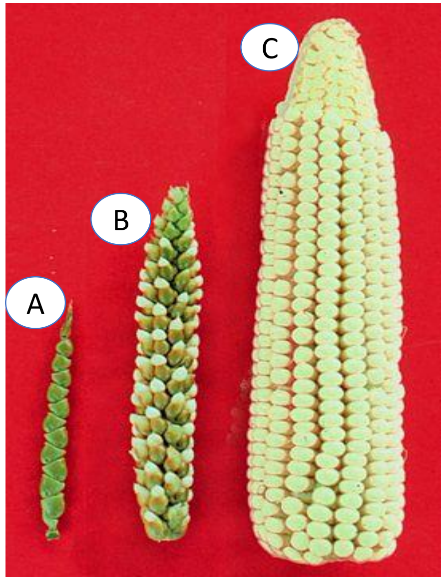


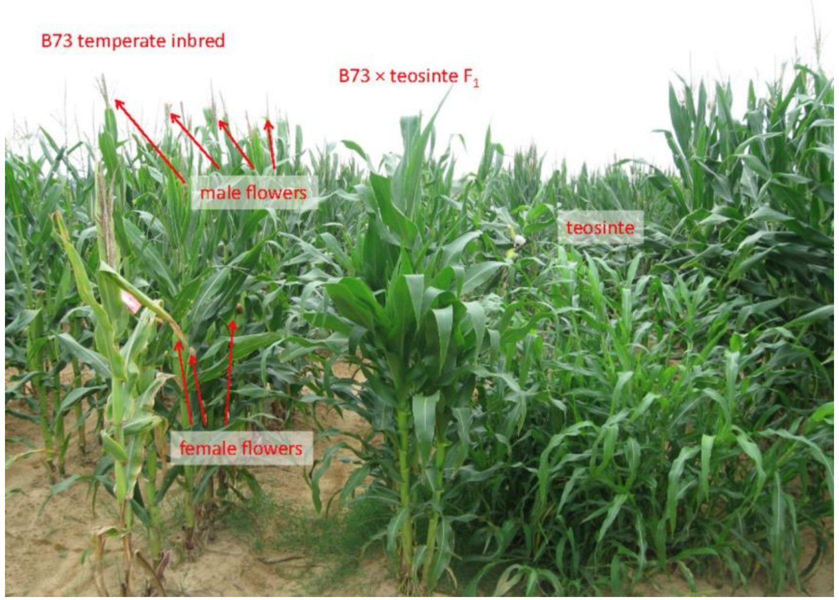
Donkey - 62 chromosomes

Mule - 63 chromosomes

Hinny - 63 chromosomes

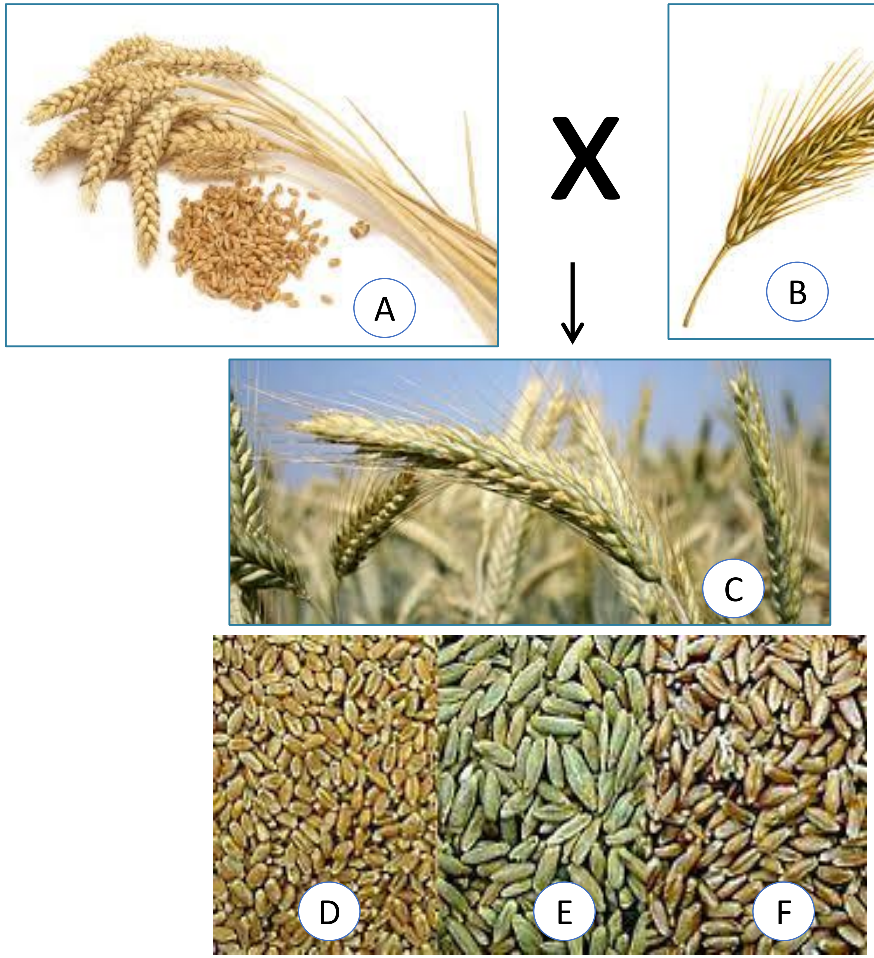
Wide crosses in plants





A – Teosinte. B – Maize-Teosinte hybrid. C – Maize

Wheat x Rye = Triticale



- **Other examples:**
  - Wheat x Maize
  - Sorghum x Saccharum
  - Sorghum x Johnson grass
    - **Homozygous recessive *iap* gene** identified in sorghum *(Laurie and Bennette, 1989)*
  - Maize x sorghum (*Mock and Loescher, 1973; James, 1977; Laurie and Bennette, 1989*) – **NOT SUCCESSFUL**

A – Wheat. B – Rye. C – Triticale. D – Wheat grain. E - Rye grain.
F - Triticale grain — triticale grain is significantly larger than that of wheat.

The Reciprocal maize x sorghum cross (Bombom unpublished data)

B maize (Female)

X

H/G/ sorghum (Male)

F1 ear at blister stage

F1 ear degenerated in 8-10 days

Maize x sorghum cross - 4th attempt (Bombom unpublished data)

Maize

X

Sorghum

Putative F1 seed

Putative F1 Plants

Selfing

X

F2 ears harvested from F1 putative "maighum" progeny plants

Pearl millet x maize crosses (Bombom unpublished data)

Pearl millet (*Pennisetum glaucum*); n=x=7

X

Maize (*Zea mays*); n=10

Pearl millet x Maize F1

Control

What is “sormaize”?

- Cross between sorghum and maize

Sorghum n=10

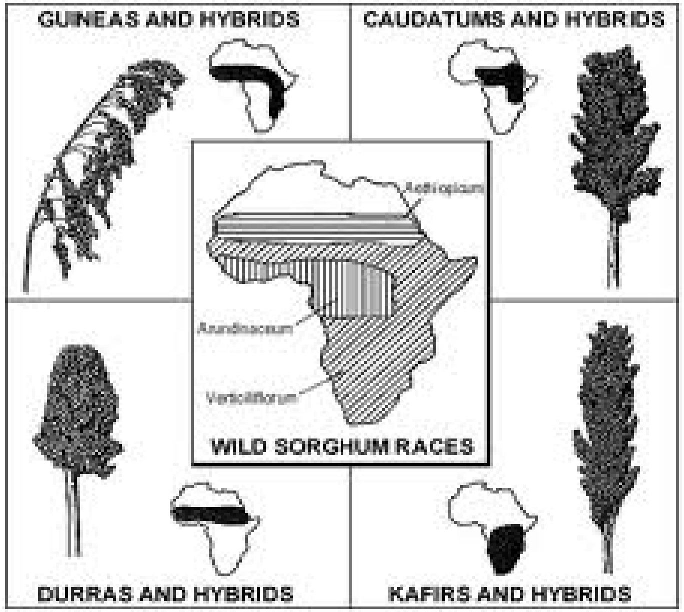
Maize n-10

Motivating factor(s) for ”Sormaize”:

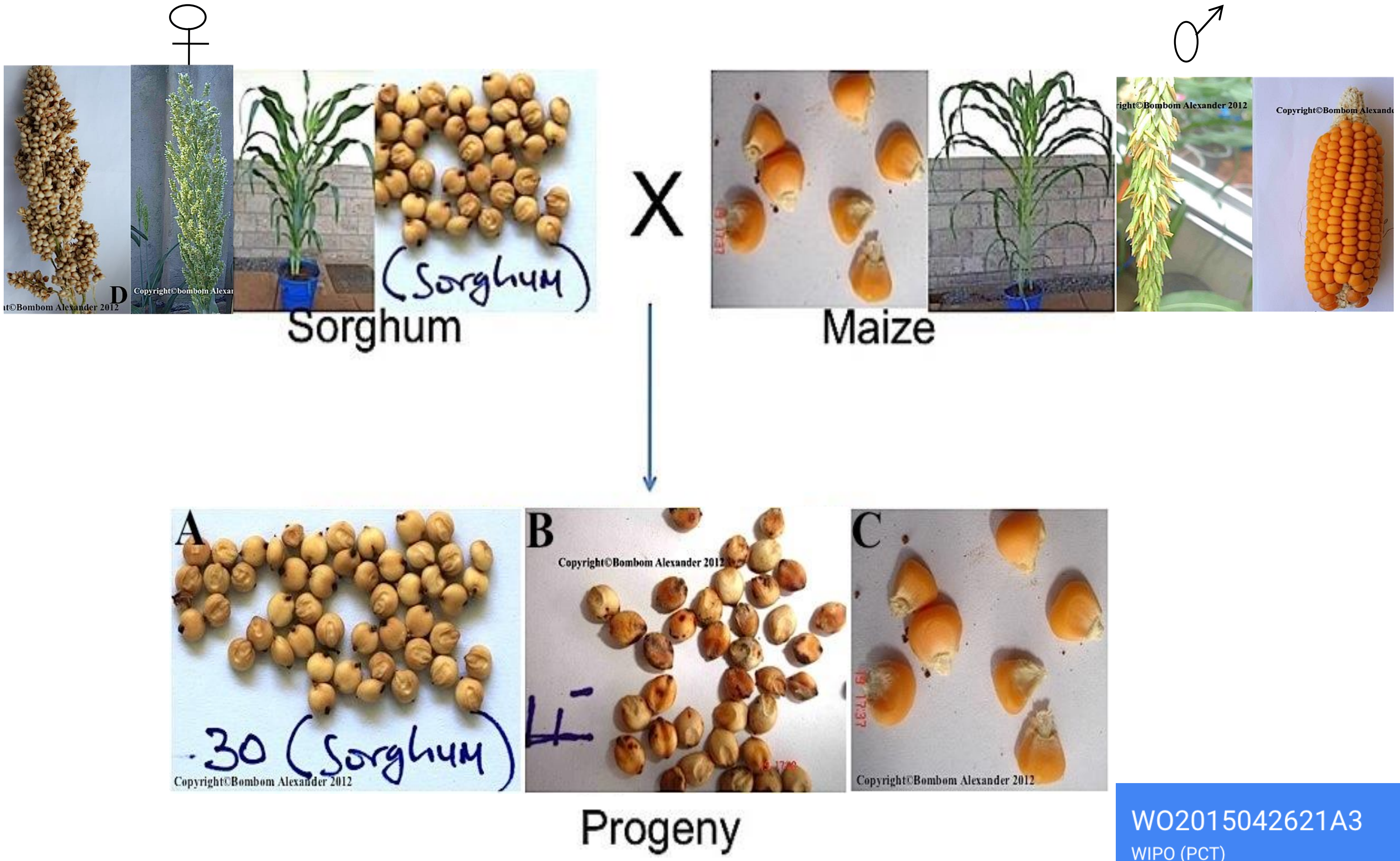
Native, low value crop adapted with our farming systems

- To **provide a novel source of increased genetic diversity and present opportunity for improvement of either one of the two crops**

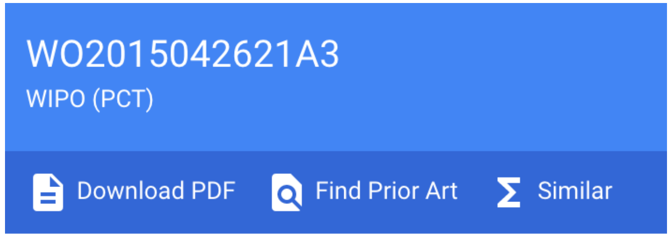
- Challenges addressed –
  - Climate resilience
  - Technology access (improved climate smart seed)
  - Low on-farm incomes
  - Markets and value addition – specialty lines



The Innovation: Sorghum x maize cross - Application PCT/AP2013/000002



https://patents.google.com/patent/WO2015042621A3/en



Methods and results

1. ♀ Sorghum X Maize ♂

↓

Putative F1 Hybrid **F1**

Selfing (X)

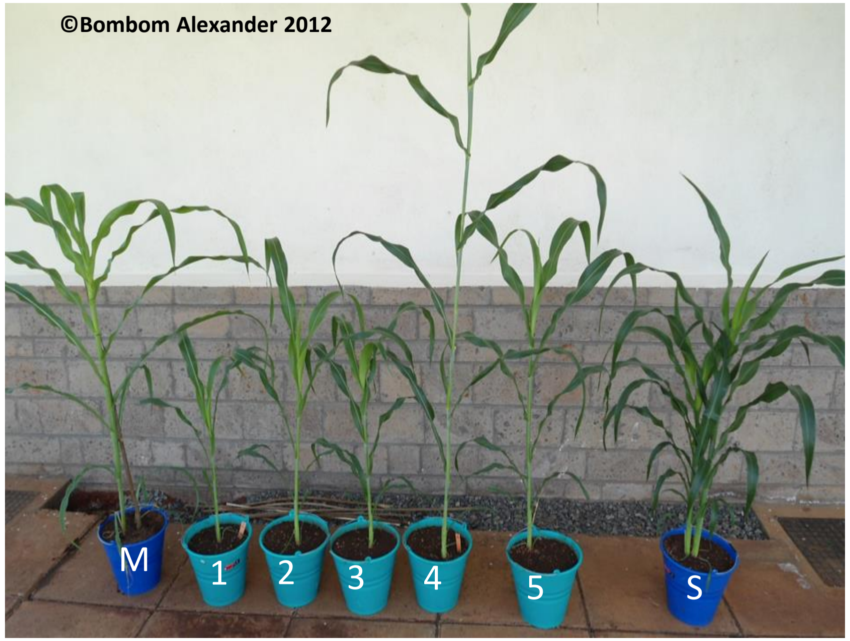
↓

Putative F2 Hybrid

Selfing of segregating F2 individual plants (X)

↓

Putative F3 Hybrid



M= Maize, S= Sorghum and 1-5 are F1 plants

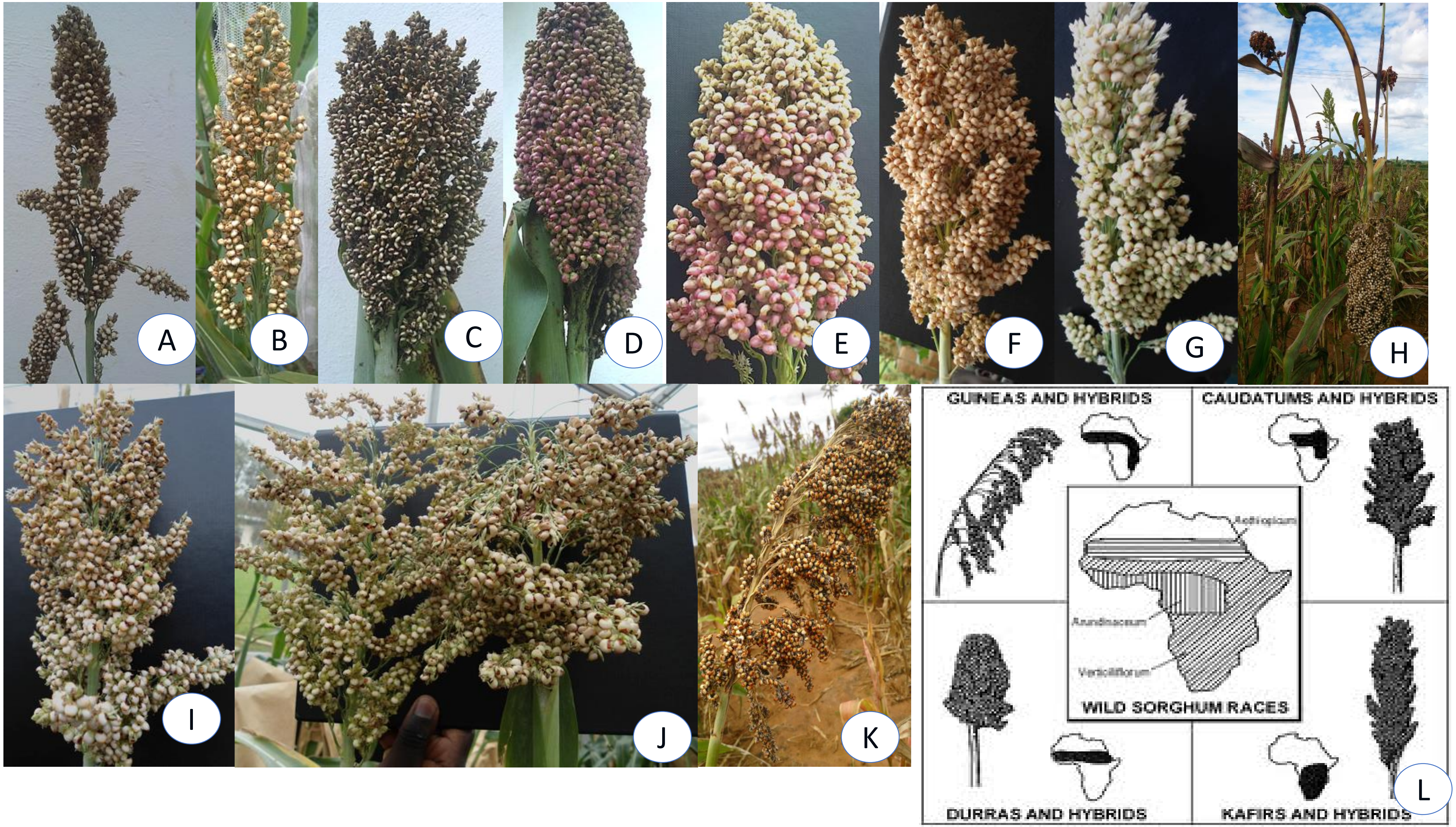
Phenotypes of original sorghum, maize and hybrid plants

D A F$_1$ F$_{2A}$ F$_{2B}$

Parents

F$_2$ plants; A = sorghum phenotype, B = Maize phenotype

F2 segregating population – fertility, color, shape and size



Segregating F2 population from this cross resemble different tribes of sorghum shown in the distribution on map

F2 plants segregating for plant stature and fertility

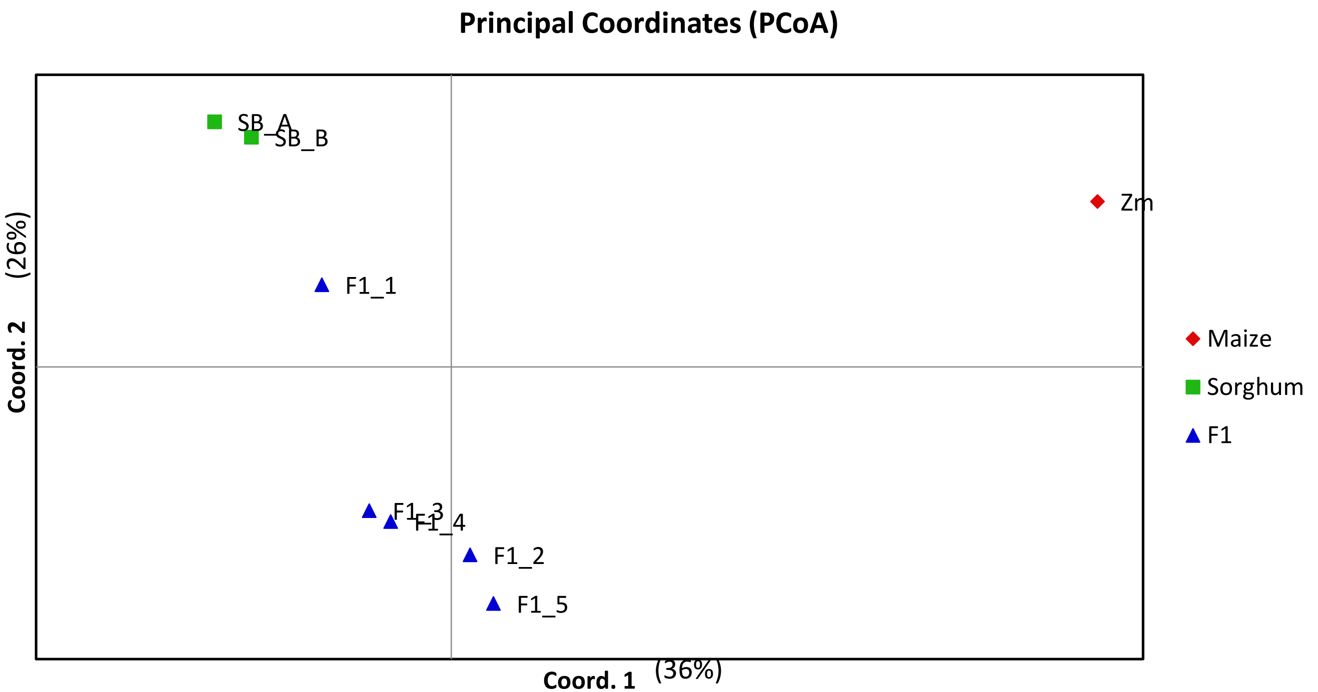
F2 plants derived from seed obtained from the same F1 panicle segregating for plant stature to resemble either the maize parent or the sorghum parent plant.

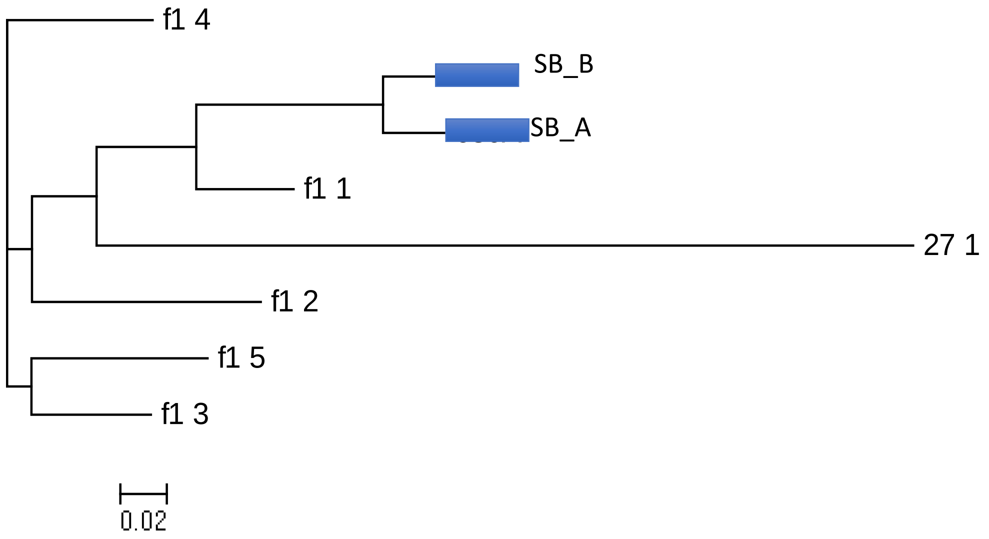
Phenotypic stability of F3 population

Sorghum like plant architecture

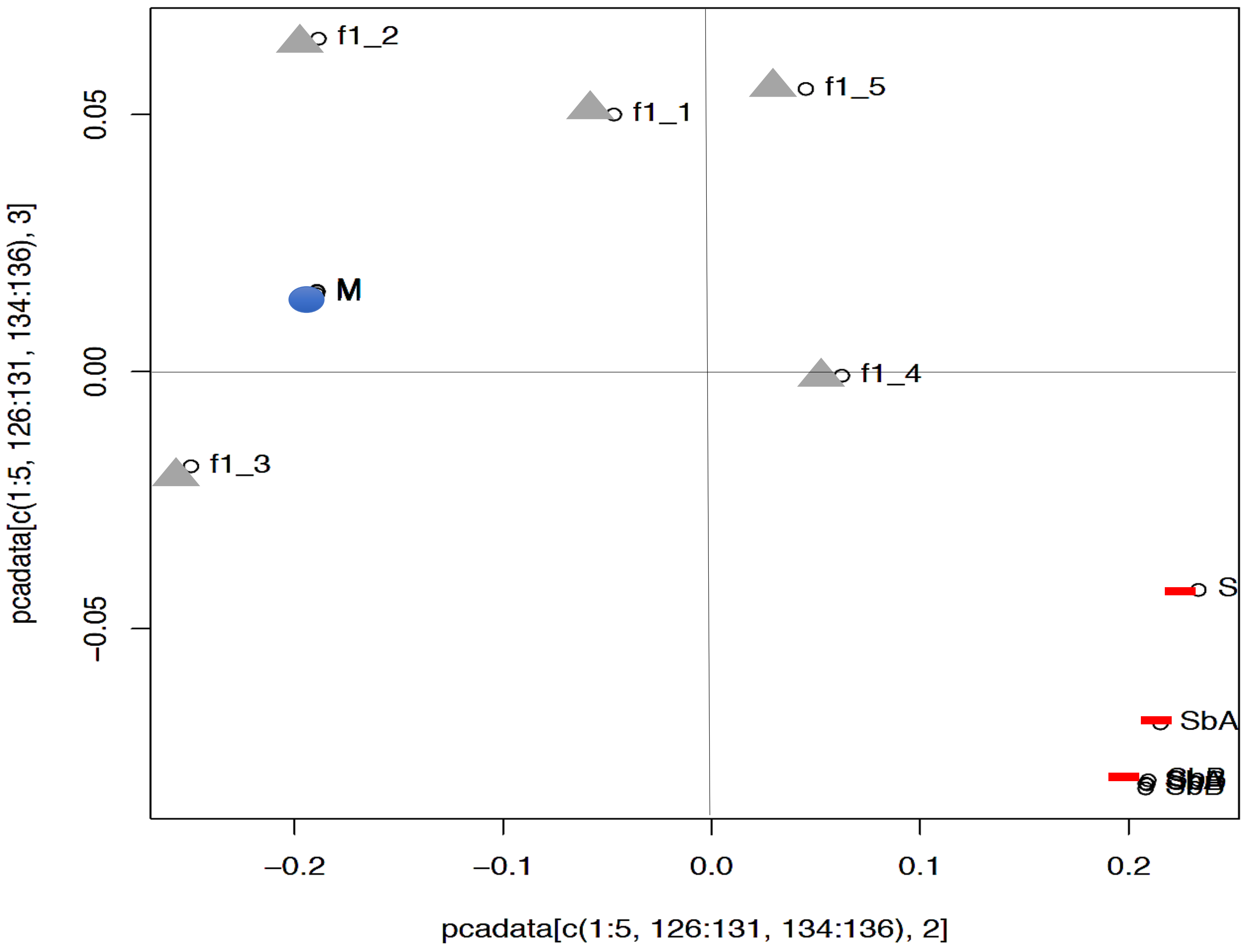
Maize like plant architecture

Results – Confirming hybridity (SSR)





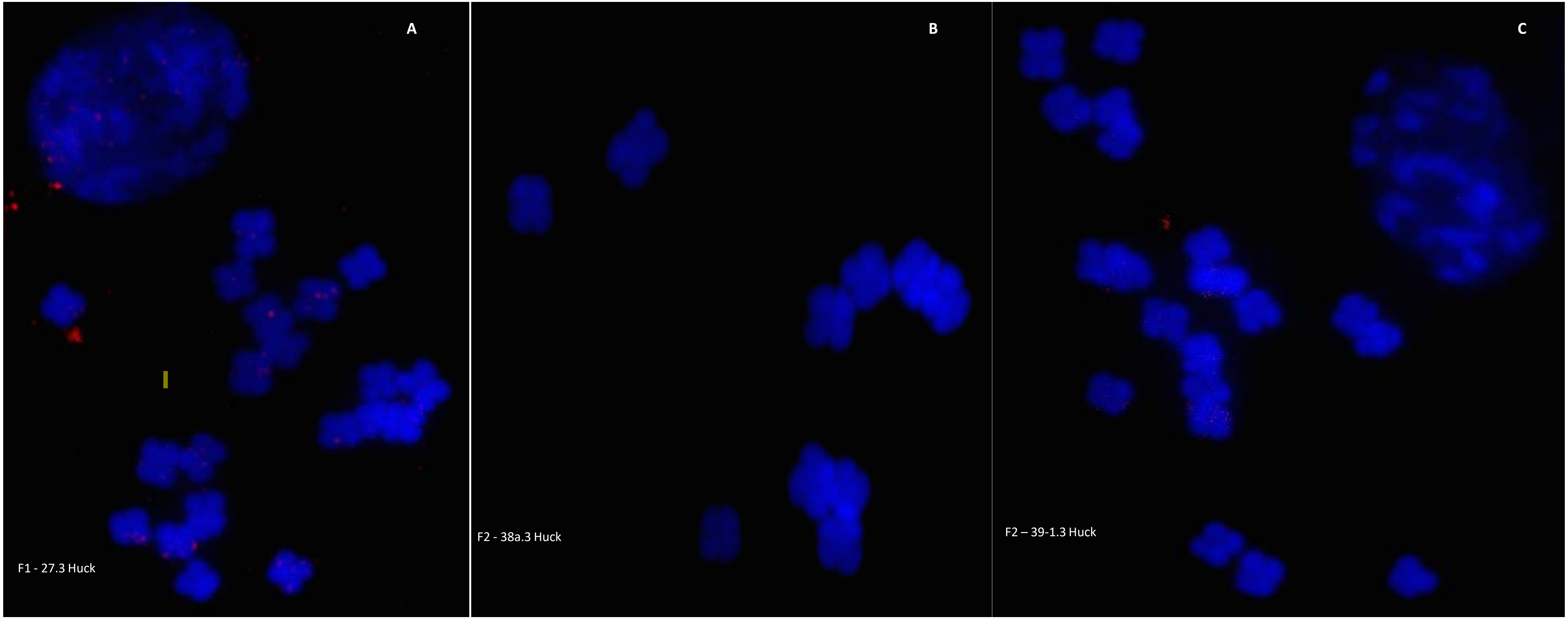
Results – Confirming hybridity (DArTSeq GBS)



Maize introgressions into sorghum – candidate genes

	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
5	*	*	*	*	*	*	*	*	*	*	*	*	*	*			
6	*	*	*	*	*	*	*	*	*	*	*	*	*	*			
7	*	*	*	*	*	*	*	*	*	*	*	*	*	*			
8	LowComlexitySeq	Chrom_Sorghum_v8	ChromPos_Sorghum_v8	AlnCnt_Sorghum_v8	AlnEvalue_Sorghum_v8	Chrom_Maize_v8	ChromPos_Maize_v8	AlnCnt_Maize_v8	AlnEvalue_Maize_v8	AdapterPos	NumPresent	HighestCount	CountSum	AvgNonZero	maizeParentsSum	sorghumParentSum	sorghumxMaizeMaxCount
9	0		0	0	999		0	0	999	22	207	67	4083	19.72464	597	0	22
10	0		0	0	999		0	0	999	20	210	70	1558	7.419048	205	0	20
11	0		0	0	999		0	0	999	20	157	89	3196	20.35669	197	0	7
12	0		0	0	999		0	0	999	20	192	38	961	5.005208	140	0	6
13	0	chromosome_10	14400202	1	3.06E-27	9 chromosome:AGPv2:9:1:156750706:1 chromosome 9	38285741	1	8.36E-27	0	247	16	1055	4.271255	197	0	3
14	0		0	0	999		0	0	999	0	196	35	1228	6.265306	172	0	3
15	0	super_59	208295	1	2.36E-28	Pt chromosome:AGPv2:chloroplast:1:140384:1 chromosome chloroplast	111125	1	1.80E-28	0	225	40	2307	10.25333	84	0	40
16	0	super_3210	1804	1	3.17E-21	6 chromosome:AGPv2:6:1:169174353:1 chromosome 6	11192858	14	6.66E-22	60	58	16	108	1.862069	5	0	16
17	0	super_59	15245	1	6.57E-29	Pt chromosome:AGPv2:chloroplast:1:140384:1 chromosome chloroplast	15305	1	1.80E-28	0	267	24	1634	6.11985	24	0	5
18	0	chromosome_3	38402982	1	4.54E-19	Pt chromosome:AGPv2:chloroplast:1:140384:1 chromosome chloroplast	96007	2	1.23E-18	55	143	10	308	2.153846	5	0	5
19	0		0	0	999	7 chromosome:AGPv2:7:1:176764762:1 chromosome 7	1.59E+08	1	2.46E-13	41	206	108	10759	52.22816	1894	0	4
20	0	chromosome_2	73058626	1	1.42E-25	7 chromosome:AGPv2:7:1:176764762:1 chromosome 7	1.65E+08	1	3.89E-25	57	203	13	620	3.054187	125	0	4
21	0		0	0	999		0	0	999	20	20	47	76	3.8	4	0	4
22	0		0	0	999	8 chromosome:AGPv2:8:1:175793759:1 chromosome 8	1.72E+08	1	2.14E-14	43	194	197	23968	123.5464	2344	0	3
23	0	chromosome_3	69863579	1	6.41E-10	3 chromosome:AGPv2:3:1:232140174:1 chromosome 3	1.69E+08	2	3.60E-11	39	154	126	7252	47.09091	638	0	3
24																	

Cytology - “sormaize” hybrids



Diploid cell | Haploid cell | Diploid cell

- Fluorescent in situ hybridization (FISH) – revealed both restored diploid and haploid plants where sorghum was female parent.

Flow cytometry - F1 and parents

Mean Nuclear DNA content of maize, sorghum and putative “sormaize” F1 hybrids as measured by flow cytometry

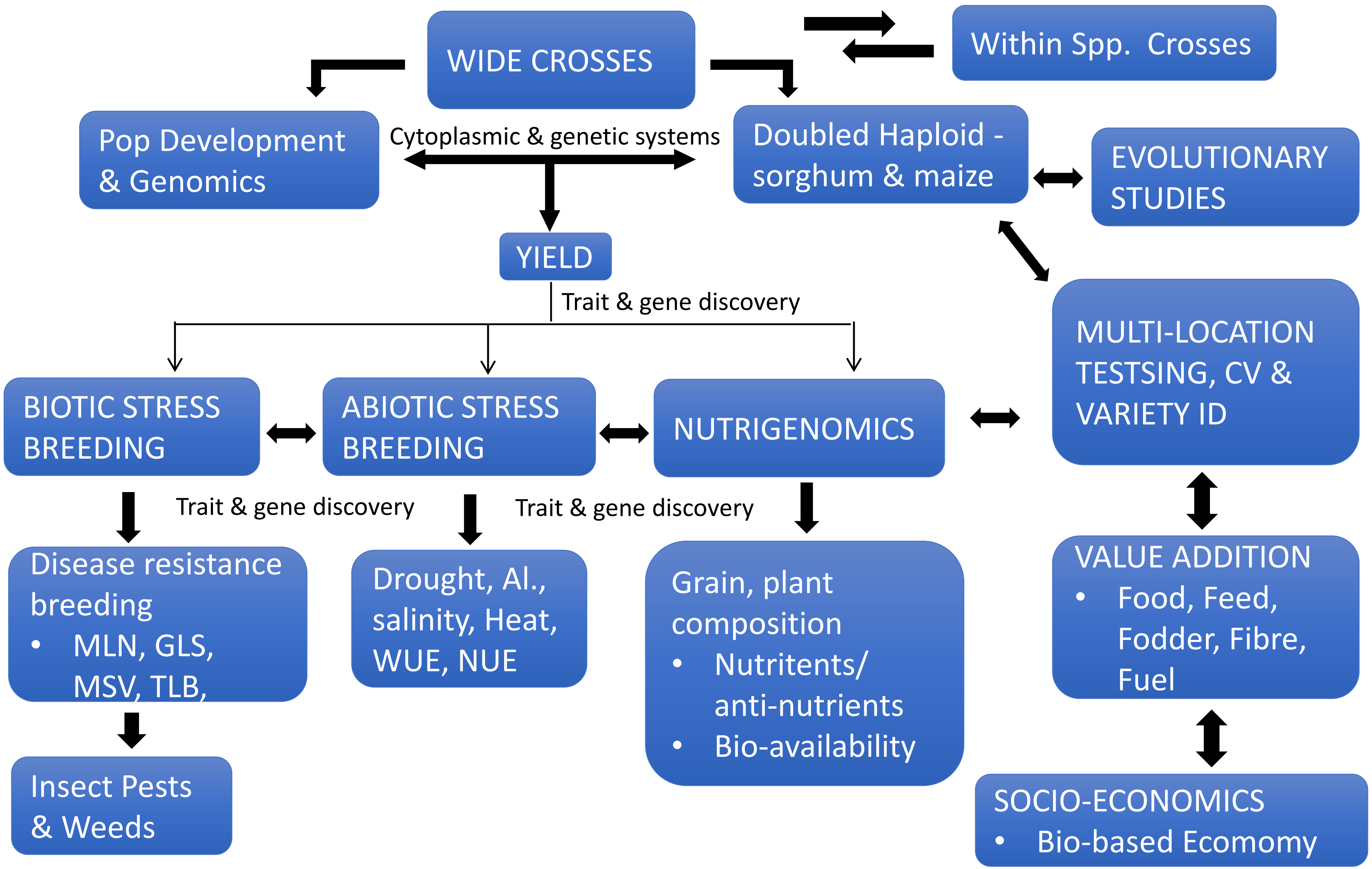
Species	DNA Content (in pg)	Mean
Zea mays	2.96	3.06±0.10
Sorghum bicolor (A line)	0.90	0.87±0.03
Sorghum bicolor (B line)	0.86	0.87±0.03
Putative “Sormaize” Hybrid	0.87	0.88±0.02

Royal Botanical Garden Kew DNA C-values. *Glycine max was used as known standard in determining DNA content for maize. Brachypodium BD 21 was used as the known standard in determining DNA content for sorghum and putative hybrid plants.* 1C, pg *Sorghum bicolor* = 0.75; *Zea mays* = 2.73. *Glycine max* = 1.13. *Brachypodium BD 21*=0.36

Summary and implication of results

- Wide crosses between maize and sorghum possible
- Sorghum x maize cross = Homoploid hybrids
- Maize x sorghum cross = Diploid, aneuploid, allotetraploid hybrids
- Enables selection in population in contrast to existing doubled haploid breeding systems
- Maize x sorghum cross increases genetic variation with potential for development of novel species.

What is possible? – Program focus areas



Good science? Yes. So what?

- Does science pay?
- **Can we harness science for social impact, environmental sustainability and poverty alleviation?**
- It is not good enough to publish

Why maize? Why sorghum?

Sorghum (2n=20)

Maize (2n=20)

- Top 5 most important crops globally
- Maize ~1.2 billion people in SSA and Latin America
- Maize market size USD 75 Billion 2022 with CAGR of 5% in period 2022 to 2032.
- Sorghum~300 million people in SSA; largely underutilized subsistence crop
- Sorghum grain & Sorghum Seeds Market size estimated at $11.8 billion as of 2021.
  - **CAGR to grow at a of 3.8%** over the forecast period 2022-2027 - https://www.industryarc.com/Research/Sorghum-And-Sorghum-Seeds-Market-Research-504917

Why maize? Why sorghum?

- Sorghum grain Market Segmentation
  1. **By Type-** Grain Sorghum, Forage Sorghum, Biomass Sorghum, Sweet Sorghum.
  2. **By Application form-** Gram Flour, Popped, Flake, Puffs, Chips, Pasta, Syrup, Others.
  3. **By End User-** Human Food/Feed, Biofuel, and Ethanol, Livestock Feed, Food Products Manufacturing, Beverage Manufacturing, Sorghum Planting, Others.
  4. **By Geography**- North America, Europe, Asia Pacific, South America, the Middle East, and Africa.

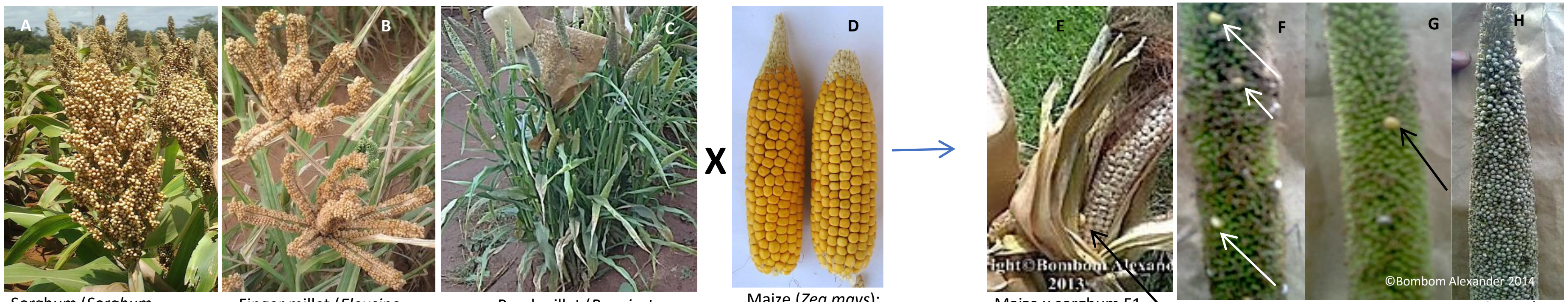
- Global sorghum seed market is projected to register a CAGR of 4.4% during the forecast period (2020-2025) https://www.mordorintelligence.com/industry-reports/sorghum-seeds-for-sowing-market

- Segmentation of the Sorghum Seeds Market
  - Open pollinated varieties and
  - Hybrids

Applications in agricultural development

Expeditious development of improved high yielding varieties

Drought tolerance and Disease resistance breeding



Sorghum (*Sorghum bicolor*); n=10

Finger millet (*Eleusine coracana*); n=9

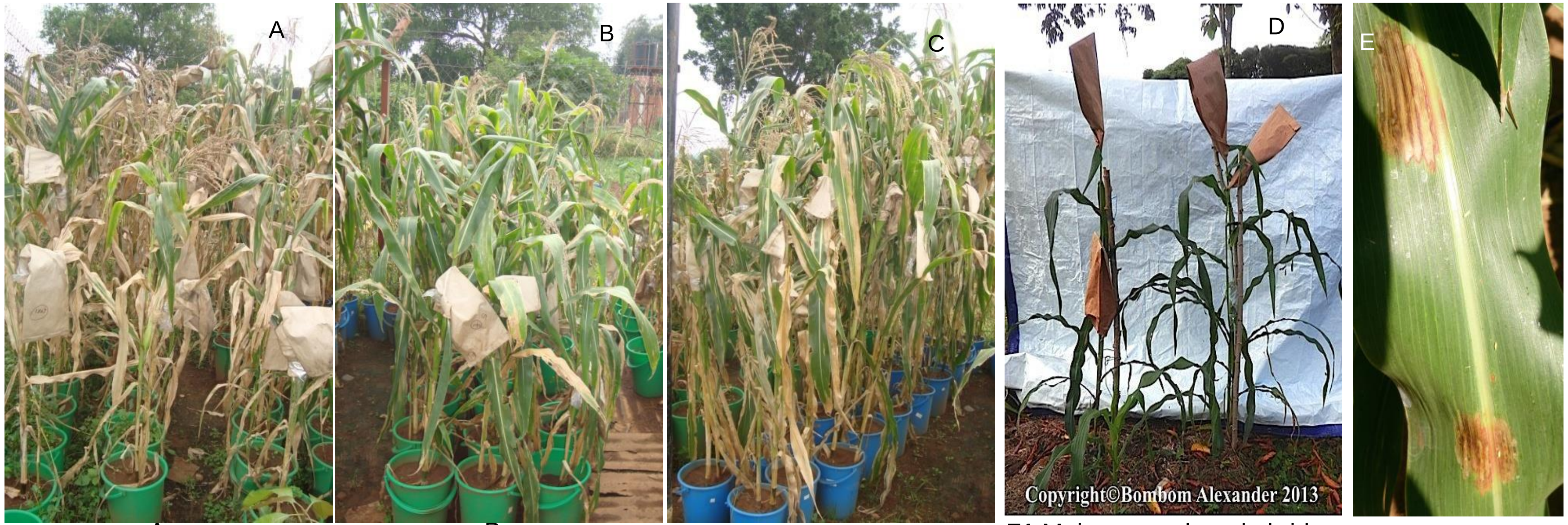
Pearl millet (*Pennisetum glaucum*); n=x=7

Maize (*Zea mays*); n=10

Maize x sorghum F1

Pearl millet x Maize F1

Control



A

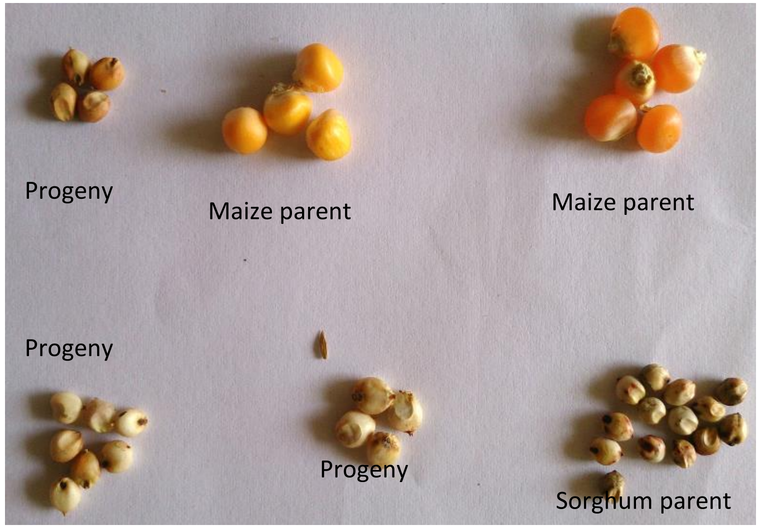
B

A x B (F1 maize hybrid)

F1 Maize x sorghum hybrid

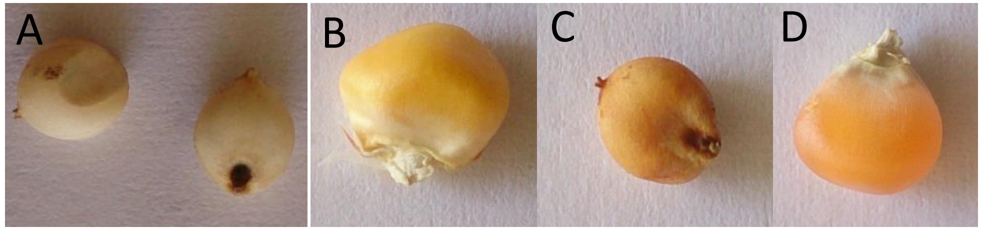
F1 Sorghum x maize hybrid

Grain and biomass yield and quality



Select directly for grain yield and biomass from segregating “sormaize” populations.

Breeding for nutrition value and grain quality



- Beta carotene introgression into sorghum – potted experiments
- Other micronutrients including zn, Fe, vit. E

Genotype	Description	Starch yield	AM (BV)	Paste clarity	Reducing sugars
A	INBRED –maize	22.8	0.06	25.0	4.5
C	INBRED –maize	39.8	0.254	8.1	3.2
B	INBRED –maize	19.9	0.208	6.7	1.5
D X B	F1 (maize x sorghum)	53.5	0.027	48.1	1.5
D	INBRED -sorghum	44.0	0.026	22.6	1.8
F (ctrl)	INBRED -sorghum	35.0	0.355	8.2	9.5
H	INBRED -sorghum	36.3	0.053	28.2	12.3
H X F (ctrl)	F1 (sorghum cross)	47.6	0.193	6.1	6.2

waxy Non waxy

Starch attributes for maize and sorghum parental lines and potential crossesAM (BV) = amylose content measured in terms of the blue value.

Preliminary data for select quality parameters

Sugar cane standard = 25

Plot	Rep	Entry	Plot No	Status	Plant no	Juice content(ml)	Brix %
1028	1	33	314	Progeny	1	J	20
1028	1	33	314	Progeny	2	J	18
1028	1	33	314	Progeny	3	J	22.3
1028	1	33	314	Progeny	4	J	20.6
1028	1	33	314	Progeny	5	J	23.2
1028	1	33	314	Progeny	6	J	20.7
1028	1	33	314	Progeny	7	J	20.7
1028	1	33	314	Progeny	8	J	23
1028	1	33	314	Progeny	9	J	21
1028	1	33	314	Progeny	10	J 21.2	22.1
1053	1	108	SB 3039(5)	Sorghum Parents	1	J	16
1053	1	108	SB 3039(5)	Sorghum Parents	2	J	16.1
1053	1	108	SB 3039(5)	Sorghum Parents	3	J	15
1053	1	108	SB 3039(5)	Sorghum Parents	4	P	16.7
1053	1	108	SB 3039(5)	Sorghum Parents	5	J	16.8
1053	1	108	SB 3039(5)	Sorghum Parents	6	J	17.6
1053	1	108	SB 3039(5)	Sorghum Parents	7	J 17.3	19.7
1053	1	108	SB 3039(5)	Sorghum Parents	8	J	18.6
1053	1	108	SB 3039(5)	Sorghum Parents	9	J	17.9
1053	1	108	SB 3039(5)	Sorghum Parents	10	J	18.2

Characterisation, value addition, outlets – food and bio-based products

1. Brew
2. Confectionery
3. Food
4. Snacks/ Pop
5. Malt/ Beverages

Grain

Stalks

Fodder/ Forage

Syrup

Compost fertiliser

Biodegradable pots

Composite panels

CONTRIBUTION TO CONFECTIONERY, FOOD AND SNACK PRODUCTS – 2 candidate lines

Outlet – Confectionery and snacks (Newman Foods) – sorghum syrup and extruded flours

SORGHUM SYRUP

Your perfect vegan sweetener with nutritional value

A project of Bomvitae Agro Industries Limited (BAIL) supported by the Bioinnovate Africa Programme.
Sorghum syrup is a biobased product derived from “sormaize” a novel sorghum cultivar developed by cross pollinating sorghum with maize (PCT/AP2013/000002).

SORGHUM – THE SMART CROP

Sorghum is native to Africa, farmers are familiar with its cultivation, is open-pollinated, environmentally sound, pest & drought tolerant, gluten-free food with non-GMO properties, supports local production and on-farm processing, is community friendly - keeps income within communities, needs no chemical inputs to thrive, is rich in antioxidants and hard-to-find nutrients: iron, calcium and potassium.

WASTE TO PROFIT

Stalks to Juice: 50kgs sorghum stalks yield 10L of raw juice.
Syrup per acre: 500 Litres.
Syrup to Profit: Current market prices are UGX. 30,000/kg = $8.43/kg.

FACTS

Proximate	Amount Per 100g	Contribution to Health benefits
Protein	1.68 g	Building blocks of organs, muscles, skin, and hormones.
Ash	7.15 g	Indicative of total amount of minerals present in food
Sucrose	12.19 g	Energy source for organs and cells, stronger immune system and improves gut health
Glucose	37.24 g	
Fructose	45.22 g	
Fibre content	0.09 g	
Antioxidant activity	136.71 mg (TE)	Protects cells against free radicals
Total phenolics	221.85 mg (GAE)	
Minerals		
Aluminium (Al)	1.86 mg	Provides a safe barrier to bacteria and contamination in food preservation
Calcium (Ca)	76.60 mg	Formation and maintainence of strong bones, teeth, cells, tissue and proper muscle function and metabolism of carbohydrates and fats
Phosphorus (P)	300.35 mg	
Cobalt (Co)	0.17 ug	Helps absorb and process vitamin B12
Copper (Cu)	0.17 mg	Maintains healthy bones, blood vessels, nerves, and immune function. Contributes to iron absorption.
Magnesium (Mg)	89.55 mg	Supports muscle, nerve function and energy production
Manganese (Mn)	1.13 mg	Helps form connective tissue, bones, blood clotting factors, sex hormones.
Iron (Fe)	3.30 mg	Helps prevent nutritional anemia and increase resistance to infection.
Potassium (K)	898.58 mg	Helps regulate fluid balance, muscle contractions and nerve signals.
Zinc (Zn)	0.98 mg	Helps immune system and metabolism function
Vitamins	Amount Per kg	
Water soluble vitamins		
Vitamin B1 (Thiamin)	2379 mg	Aids metabolism of carbohydrates and fat for energy.
Vitamin B2 (Riboflavin)	285 mg	
Vitamin B5 (Pantothenic acid)	2079 mg	
Vitamin B9 (Folate)	152 mg	Essential for red and white blood cells synthesis in the bone marrow, healthy cell growth and function
Vitamin B12 (methylcobalamin)	148 mg	Essential for healthy blood and nerve cells and DNA synthesis

APPLICATIONS

1. Sweetener and preservative in confectionery and bakery.
2. Topping for waffles, pancakes, Biscuits and bread.
3. Direct consumption as nutritional supplement

BOMVITAE AGRO INDUSTRIES LIMITED
Empowering communities . Improving livelihoods
+256752368259

CONTRIBUTION TO BEVERAGE PRODUCTS - 2 candidate lines

Sorghum Beer

தமிழ்

Outlet – 1. Century Bottling Company Uganda - flour
2. Breweries Limited (UBL) – for seed and grain.

SORGHURM CODE	MOISTURE (<=12.5)	EXTRACT(>80)	FAT (<=4)	PROTEINS(<=12.5)	AFLATOXIN TEST(>=4)	General Comment
AJJ-17	12.7	79.7	3.1	11.2	1.8	Red grain
AJ-62	14	81	3	11	3.4	Brown grain
AJ-75	17.9	110.3	1.6	7.8	0	White grain: Advance to further testing in Germany; seed increase and multiplication in season 2023A and 2023B; Carry out NPT, DUS, MLT for release.
AJ-114	13.5	80.8	3.4	12.7	0	White grain: Advance to further testing in Germany; seed increase and multiplication in season 2023A and 2023B; Carry out NPT, DUS, MLT for release.
AJ-136	13.6	80.7	3.1	11.6	>30	Brown grain
AJ-135	12.7	83.6	3.1	11.2	6.3	Cream to light brown grain
AJ-91	14.8	84.1	3.2	11.3	1.7	Brown to redish grain
AJ-70	15	85	3	11.6	0	White grain
AJ-28	14.7	84.8	2.9	11.8	1.4	Brown grain
AJ-45	12.9	78.3	3.2	11.2	14	Red grain

Notes:

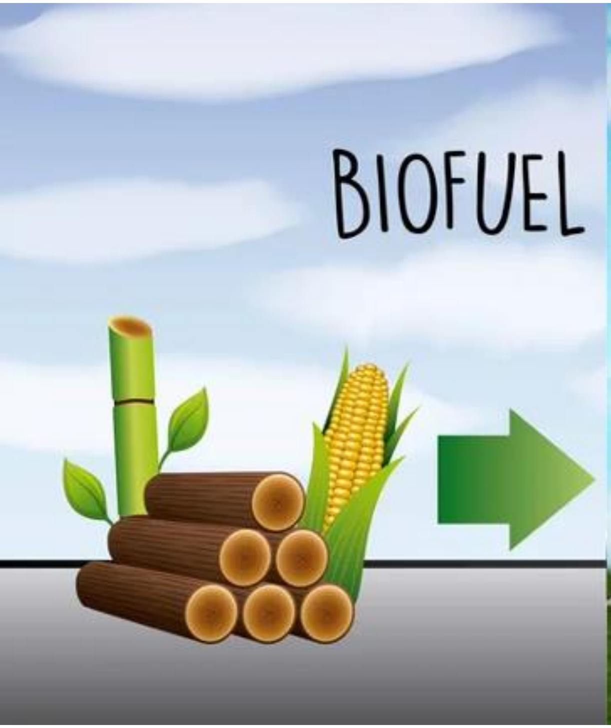
Samples highlighted in yellow are test samples that meet the brewers needs in terms of extract and have been selected for advancement; Samples in Blue are commercial lines currently being used by the brewery. Further drying needed for all.

CONTRIBUTION TO LIVESTOCK

Forage sorghum for livestock – 1 Candidate line (each hill produces 10 to 12 productive tillers)

SORGHUM FOR FODDER – 1 Candidate line

CONTRIBUTION TO CLEAN ENERGY – 1 Candidate line



Flame from bioethanol extracted from sormaize

THE REPUBLIC OF UGANDA

I SIGNIFY my assent to the bill.

Y.K. Museveni
President

Date of assent: 4/6/2018

(2) An application for a licence shall, as may be required by the licensing authority, be accompanied by—

(a) a statement of the feasibility of the business for which the licence is applied;

(b) the business plan indicating the location of the proposed business;

(c) the type of feedstock to be used to produce the biofuel;

(d) the acreage of the land to be used for the business;

(e) the technology to be used in the production, storage, transportation or blending of biofuels in petroleum products, as the case may be;

(f) the occupational health, public safety and environmental measures to be applied in the production, storage, transportation or blending, of biofuels in petroleum products, as the case may be;

(g) confirmation of compliance with the National Environment Act with regard to environmental regulation;

(h) where the application is for the production of biofuels, a certificate granted by the Ministry responsible for agriculture confirming that the feedstock to be used conforms to the ecosystem of Uganda and that the food security of Uganda will not be compromised by the proposed production;

(i) any other authorization that may be required under any other law; and

(j) the prescribed fees, which shall be paid into the Consolidated Fund.

ACCESS TO TECHNOLOGY, PRECISION AND VALUE ADDITION TOOLS

Mechanisation access

Value addition to stover - syrup

Value addition to bagasse - silage

What has been done

- Characterisation of wide cross hybrids
  - Genotyping, Phenotyping, functional diversity
- Specialty sormaize lines for industry identified
- Outlets identified – breweries, livestock, food, biofuel,..
- Value addition to small grains
- Biobased Prototypes developed
- Seed regeneration and increase
- Start up registered - BAIL →

bail
BOMVITAE AGRO INDUSTRIES LIMITED
Empowering communities . Improving livelihoods

What needs to be done

- Seed conservation and safety duplication
- NPTs, DUS and variety release of specialty “sormaize”
- Sormaize seed production and seed system (access)
- Fingerprinting of specialty varieties
- Commercialisation and licensing to commercial companies
- Biobased product development and refinement
- Market insights, testing and adoption
- Access to small scale mechanisation
- Capacity building for farmers and scientists into enterprenuers
- Capacity building of start up/ SME - BAIL
- Extend wide crosses to other cereals,
- IP – PVP/ PBR Registration

Develop AI & ML models for grain quality - High throughput phenotyping - collaborations

Genotype	Description	Starch yield	AM (BV)	Paste clarity	Reducing sugars
A	INBRED –maize	22.8	0.06	25.0	4.5
C	INBRED –maize	39.8	0.254	8.1	3.2
B	INBRED –maize	19.9	0.208	6.7	1.5
D X B	F1 (maize x sorghum)	53.5	0.027	48.1	1.5
D	INBRED -sorghum	44.0	0.026	22.6	1.8
F (ctrl)	INBRED -sorghum	35.0	0.355	8.2	9.5
H	INBRED -sorghum	36.3	0.053	28.2	12.3
H X F (ctrl)	F1 (sorghum cross)	47.6	0.193	6.1	6.2

waxy Non waxy

1. “Imagination is more important than knowledge” – Albert Einstein

2. “They thought I was crazy, absolutely mad”.
   — Barbara McClintock. *The response (1944) of the National Academy of Sciences, to her (later Nobel prize-winning) theory that proposed that genes could transition—'jumping'—to new locations on a chromosome.*

3. “If you know you're right, you don't care. You know that sooner or later, it will come out in the wash”.
   — Barbara McClintock (*When asked about the long delay in recognition for her discovery).*

4. “It never occurred to me that there was going to be any stumbling block. Not that I had the answer, but [I had] the joy of going at it. When you have that joy, you do the right experiments. You let the material tell you where to go, and it tells you at every step what the next has to be because you're integrating with an overall brand new pattern in mind”.
   — Barbara McClintock

(*When asked how she could have worked for two years without knowing the outcome).*

Acknowledgements

- The African Biosciences Challenge Fund (ABCF)
- Bill and Melinda Gates Foundation
- BecA-ILRI Hub, Birchler lab (University of Missouri), Assaf lab (Tel Aviv University (ICCI)), ICRISAT
- Demand-Led Breeding (DLB) Program

Thank you

Food and Agriculture Organization of the United Nations

The International Treaty ON PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE

Australian AID

syngenta foundation for sustainable agriculture

SWEDEN

d·lb demand-led breeding

CSIRO

BioInnovate Africa

icipe@50 1970 – 2020 Insects for Life

biosciences eastern and central africa

ILRI INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

NARO

bail BOMVITAE AGRO INDUSTRIES LIMITED Empowering communities . Improving livelihoods

BILL & MELINDA GATES foundation