



# FEED THE FUTURE

The U.S. Government's Global Hunger & Food Security Initiative



Collaborative Research  
on Sorghum and Millet

# 2022 ANNUAL REVIEW MEETING

March 21-25, 2022

*Virtual*



**USAID**  
FROM THE AMERICAN PEOPLE

**KANSAS STATE**  
UNIVERSITY



**FEED THE FUTURE**

The U.S. Government's Global Hunger & Food Security Initiative



**Collaborative Research  
on Sorghum and Millet**

# **Sorghum Trait Deployment Pipeline for Improved Food and Feed Value**

**SMIL Annual Review Meeting  
March 24, 2022**



**USAID**  
FROM THE AMERICAN PEOPLE

**KANSAS STATE**  
UNIVERSITY



## Research Team

### Purdue University

Mitch Tuinstra

Cliff Weil (**Retired**)

### INRAN

Seyni Ousmane

### ISRA/CERAAS

Elisabeth Diatta-Holgate

Cyril Diatta



## Project Objectives

- ❖ Develop sorghum varieties with improved post-cooking protein digestibility and nutritional value – Dr. Elisabeth Diatta-Holgate
- ❖ Develop forage sorghum cultivars that meet the needs for increased forage production in Niger and West Africa – Dr. Ousmane Diakite



## Objective 1: Improving protein digestibility

- Sorghum has decreased protein digestibility following wet cooking.
- Limited natural variability exist for protein digestibility.
- The *hl* locus was introduced into 4 ISRA lines (BC3 stage).
- Additional EMS mutants with improved digestibility were identified.
- New allelic variants are being explored.



## Activity 1. Develop and commercialize sorghum cultivars with improved post-cooking protein digestibility

- 1.1 Develop and advance breeding populations for highly digestible protein using hl from P721Q.
- 1.2 Introgress highly digestible protein traits from EMS mutants into elite West African cultivars.
- 1.3 Work with partners on end-product testing (bread and couscous) and in animal feeding trials (poultry).
- 1.4 Share material with regional SMIL collaborators.





## 1.1 Develop and advance breeding populations for highly digestible protein using hl from P721Q



## 1.1.1 Test highly digestible genotypes derived from (ISRA621B x P721Q)-BC3

- 4 ISRA sorghum varieties crossed to P721Q
- *In vitro* protein digestibility tests completed at CERAAS

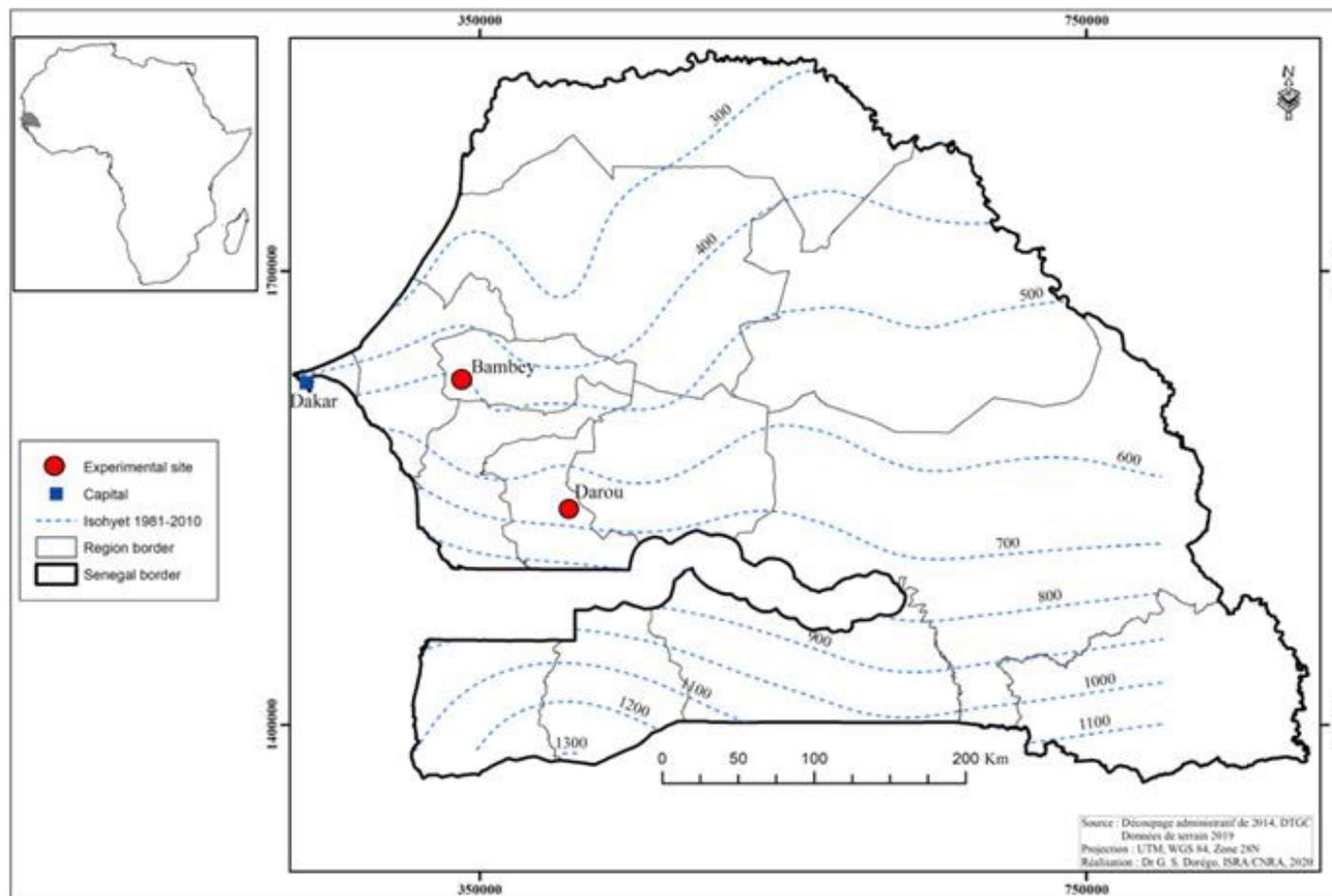
Cross	Generation	Number of HD entries	Range of digest. (%)	Increase in digest. (%)
Darou x P721Q	BC3F5	14	55.3 - 72.6	~ 29
Nguinthe x P721Q	BC3F5	7	67.5 - 79.5	~ 40
Nganda x P721Q	BC3F5	3	56.0 - 62.3	~ 20
Faourou x P721Q	BC3F6	12	59.2 - 72.2	~ 31



## 1.1.2 Backcross the *hl* locus into ISRA 621A, 622A and 622B

- 23 highly digestible lines + 5 checks were evaluated in preliminary yield trial in Bambey and Sinthiou Maleme.

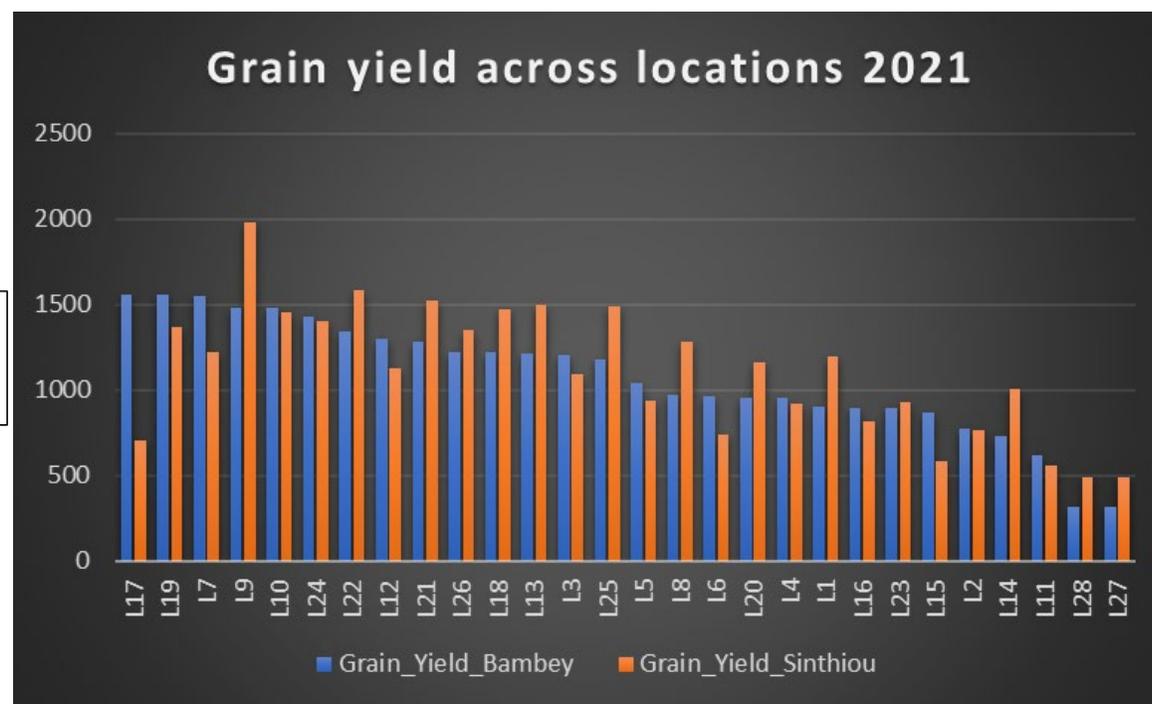
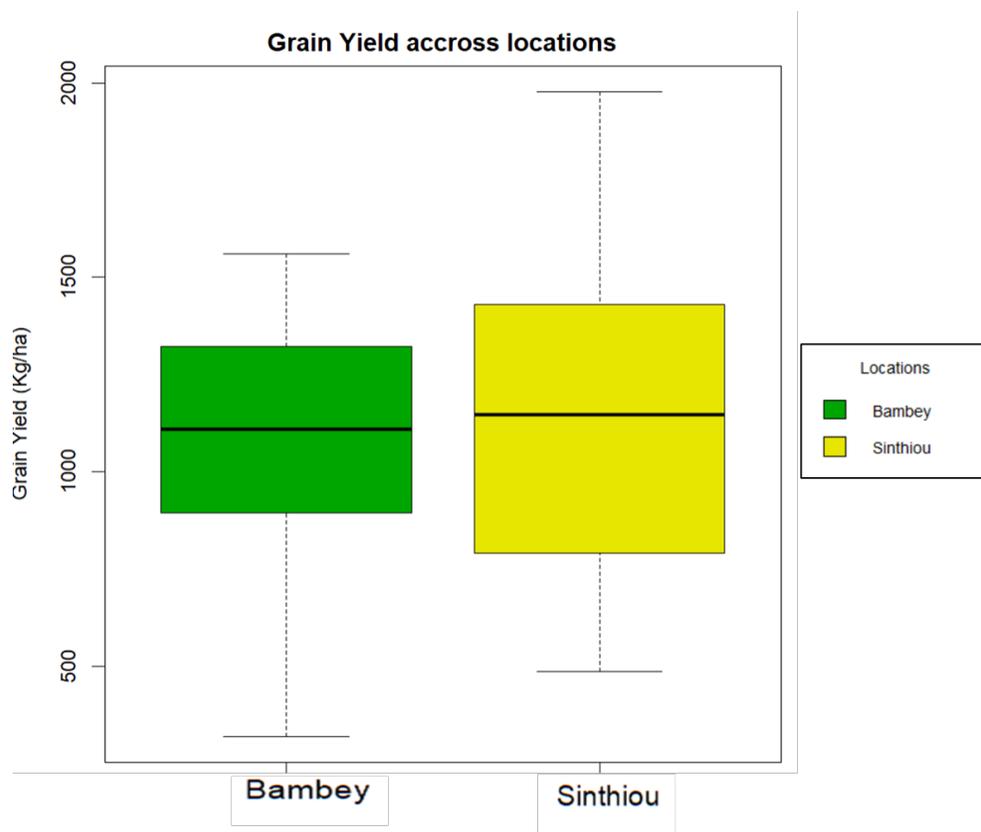
Darou x P72IQ
Nguinthe x P72IQ
Nganda x P72IQ
Sureno





## 1.1.2 Backcross the hl locus into ISRA 621A, 622A and 622B

### ■ Preliminary yield trial results





## 1.1.2 Backcross the hl locus into ISRA 621A, 622A and 622B

- The 12 best and most stable entries across 2 locations were selected for further evaluation

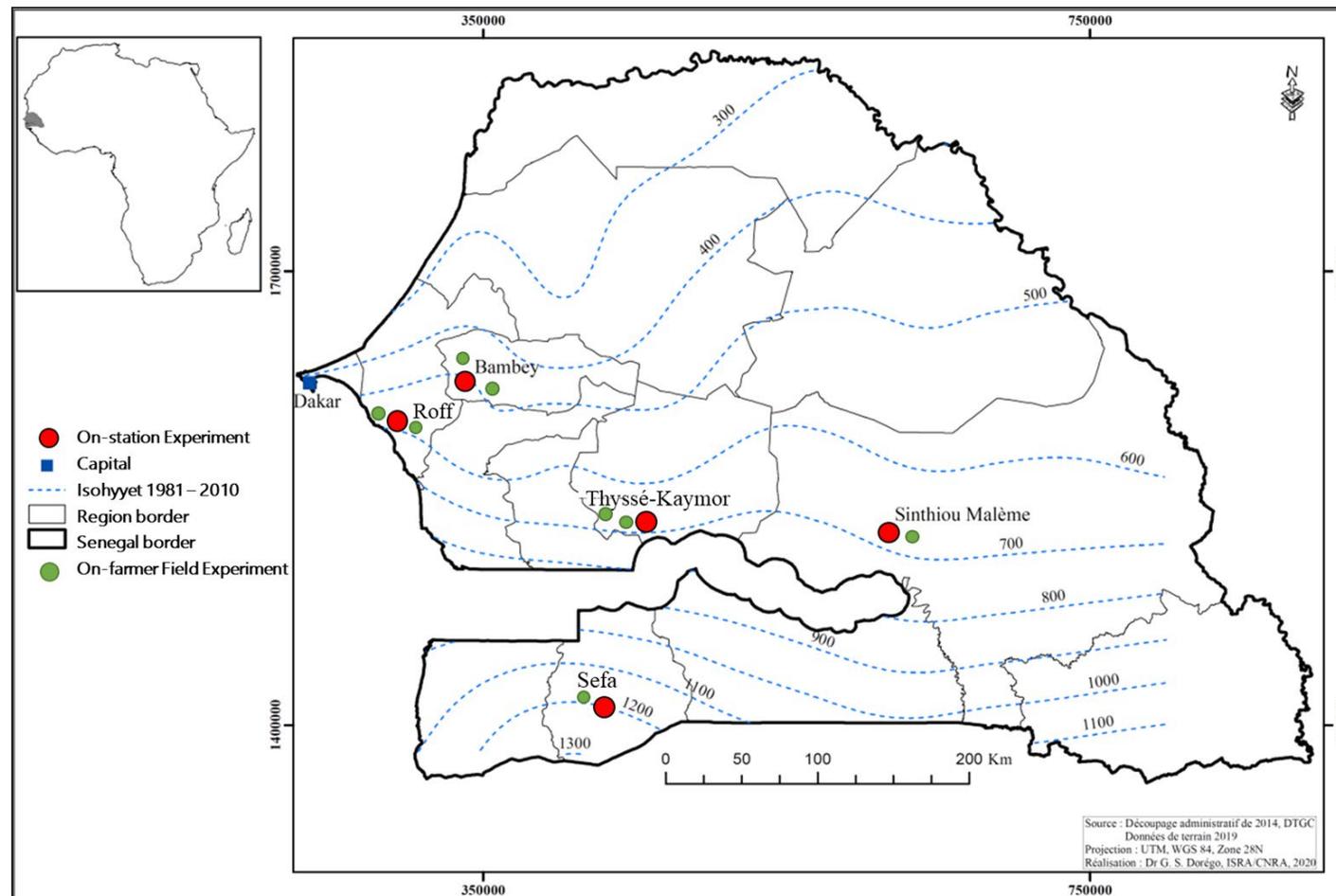
Code	Entries	Protein Digest. (%)	Grain mold score
L7	L21-1	79.1	2
L13	L42-6	75.4	3
L22	L48-10-1	66.4	4
L18	L12-9-1	66.1	4
L21	L42-6-2	66.1	3
L19	L24-10	62.6	4
L10	L13-2	62.3	4
L9	L74-1-1	60.1	3
L12	L9-1-2	55.3	4
L26	Darou	43.1	4
L25	Nganda	41.9	3
L24	Nguinthe	38.5	2
L27	P721Q	60.0	3
L28	Sureno	-	5



## 1.1.3 Test Highly Digestible Genotypes in Multi-location Field Trials

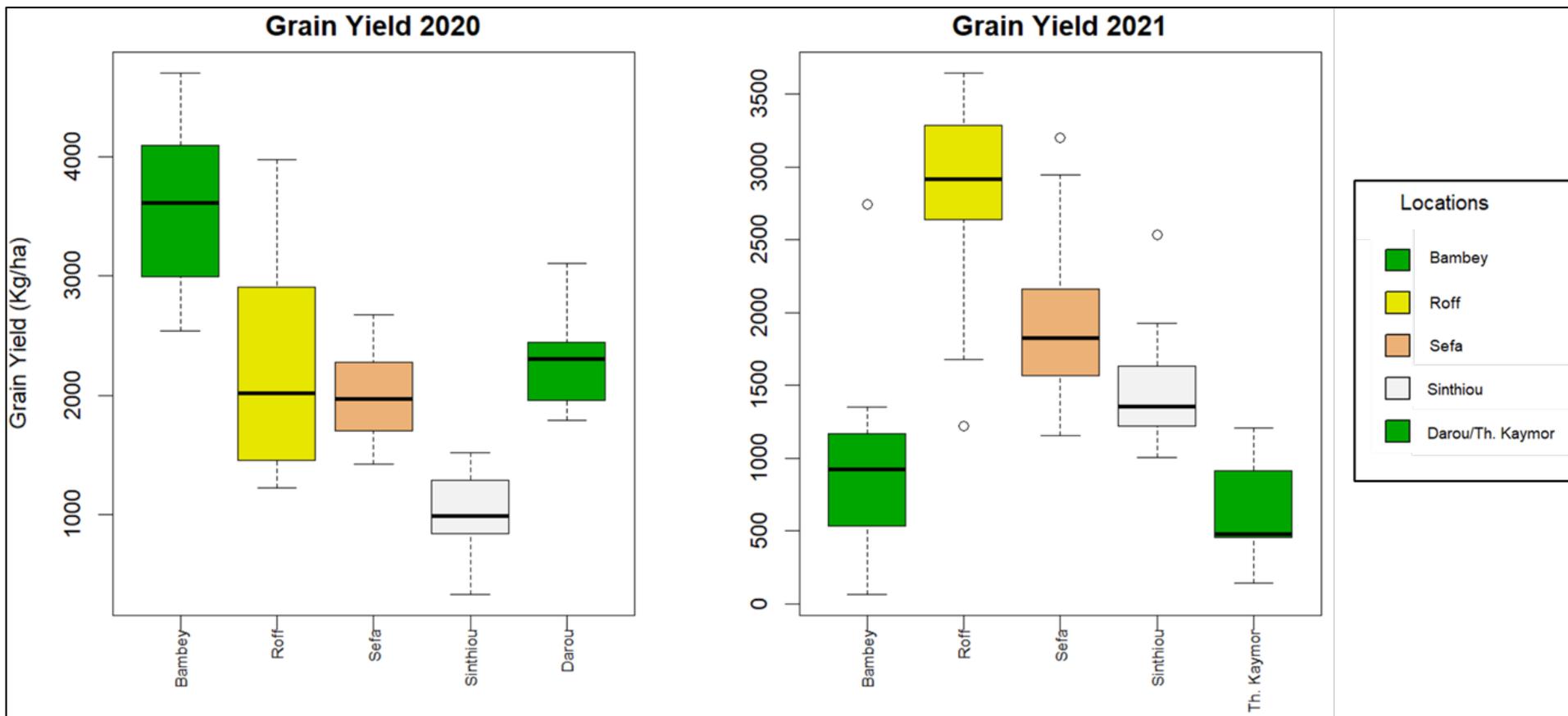
### Year 2 of field testing

- 12 highly digestible lines (Faourou x P721Q) were evaluated in multi-location trials
  - 5 research stations (red dots)
  - 8 farmers fields (green dots)





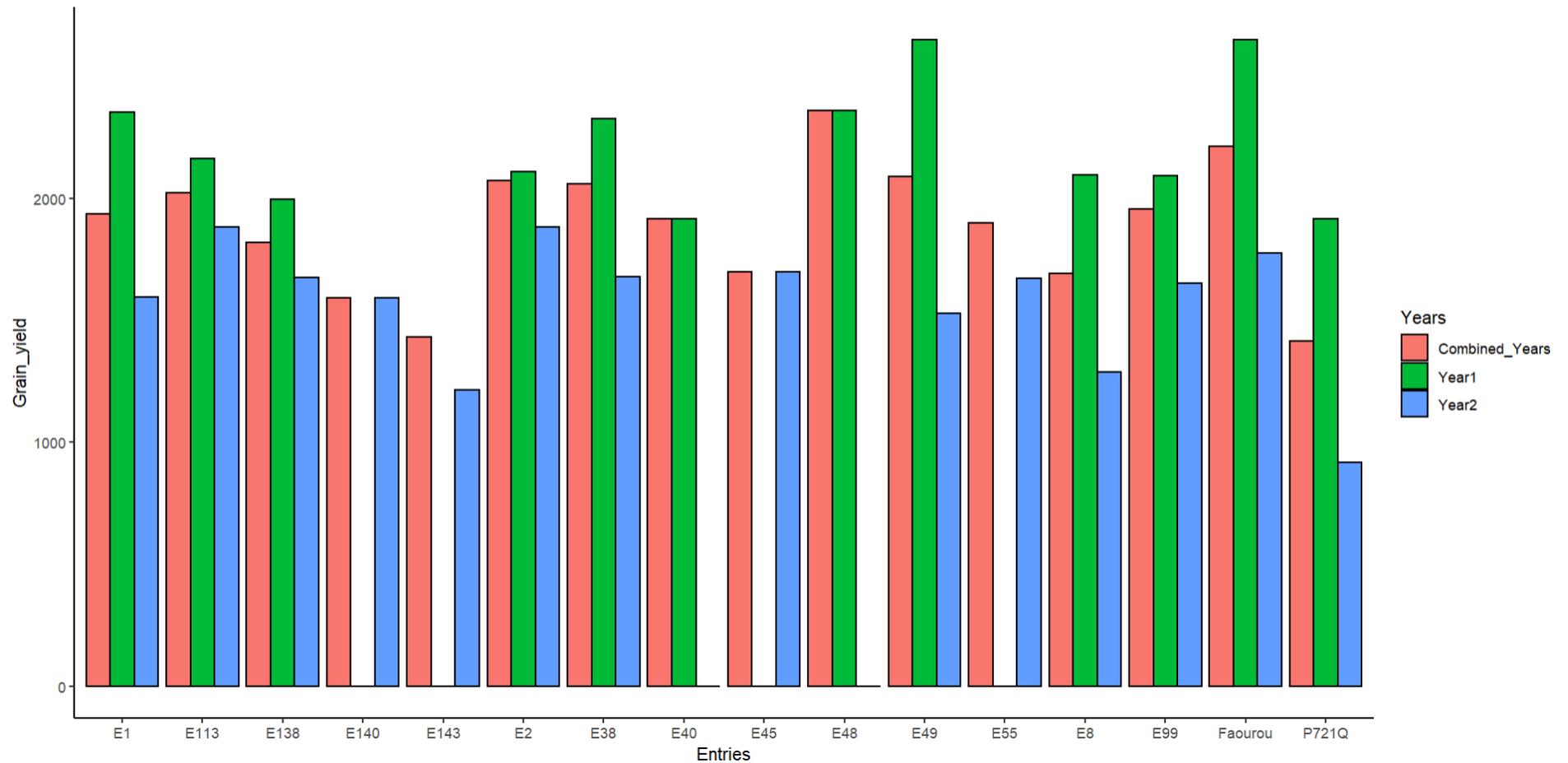
## 1.1.3 Test Highly Digestible Genotypes in Multi-location Field Trials



2021 grain yields per location were lower than 2020

## 1.1.3 Test Highly Digestible Genotypes in Multi-location Field Trials

Yield potential up to 2.6 t/ha





## 1.1.3 Test Highly Digestible Genotypes in Multi-location Field Trials

- Grain yield of HD lines were comparable to Faourou and stable across environments
- 20-31% increase in protein digestibility
- The best lines combining PD > 60% and max grain mold score of 3.

Entries	Grain Yield (kg/ha)	Protein Digest. (%)
E2	1884.25 a	61.01 a
E113	1883.62 a	65.01 a
Faourou	1775.12 ab	40.92 b
E45	1697.59 ab	65.43 a
E38	1677.95 ab	60.09 a
E138	1674.33 ab	64.22 a
E55	1673.53 ab	66.29 a
E99	1653.07 ab	64.29 a
E1	1594.56 ab	62.96 a
E140	1592.87 ab	67.35 a
E49	1530.15 ab	72.15 a
E8	1287.49 ab	59.20 a
E143	1215.09 ab	65.03 a
P721Q	917.67 b	60.66 a



## 1.1.3 Test Highly Digestible Genotypes in Multi-location Field Trials

### Participatory varietal selection (PVS)

- A PVS was done at Roff, Sefa and Sinthiou Maleme during the rainy season 2021
- A total of 45 farmers (19 males / 26 females) participated
- Lines selected based on agronomic performances and PVS will be proposed for registration in 2022





## 1.1.3 Test Highly Digestible Genotypes in Multi-location Field Trials

### PVS Results

Entries	SEFA		SINTHIU MALEME		ROFF	
	FREQUENCY	RANK	FREQUENCY	RANK	FREQUENCY	RANK
E1	1.14	3	2.00	9	1.57	8
E2	1.32	5	1.63	4	1.71	10
E8	1.14	3	2.31	13	1.00	1
E38	1.00	1	1.69	5	1.57	8
E45	2.05	10	1.56	2	1.43	6
E49	1.77	8	1.56	2	1.71	10
E55	1.50	7	1.94	8	1.00	1
E99	2.05	10	1.88	7	1.29	5
E113	1.45	6	2.13	11	1.14	4
E138	2.23	13	2.13	11	1.71	10
E140	1.05	2	2.00	9	1.86	13
E143	2.05	10	1.81	6	1.43	7
Faourou	1.86	9	1.44	1	1.00	1
P721Q	2.91	14	2.56	14	2.71	14



## 1.1.3 Test Highly Digestible Genotypes in Multi-location Field Trials

The best lines were selected based on yield, PD and PVS ranking

- Grain yield comparable to Faourou and stable across environments.
- Improved protein digestibility of 20-31%.
- 5 best lines were selected based on performance, yield, GM, and farmers' ranking.

Entries	Yield Potential (kg/ha)	Protein Digest. (%)	PVS Ranking
E38	2058.99 ab	60.09 a	1
Faourou	2213.05 a	40.92 b	2
E55	1900.33 ab	66.29 a	3
E2	2073.48 ab	61.01 a	5
E1	1937.13 ab	62.96 a	6
E113	2023.70 ab	65.01 a	7
E8	1692.09 ab	59.20 a	4
E140	1592.87 ab	67.35 a	8
E45	1697.59 ab	65.43 a	9
E49	2090.55 ab	72.15 a	10
E99	1955.32 ab	64.29 a	11
E143	1432.51 ab	65.03 a	12
E138	1820.00 ab	64.22 a	13
P721Q	1416.16 b	60.66 a	14



## Activity 1. Develop and commercialize sorghum cultivars with improved post-cooking protein digestibility

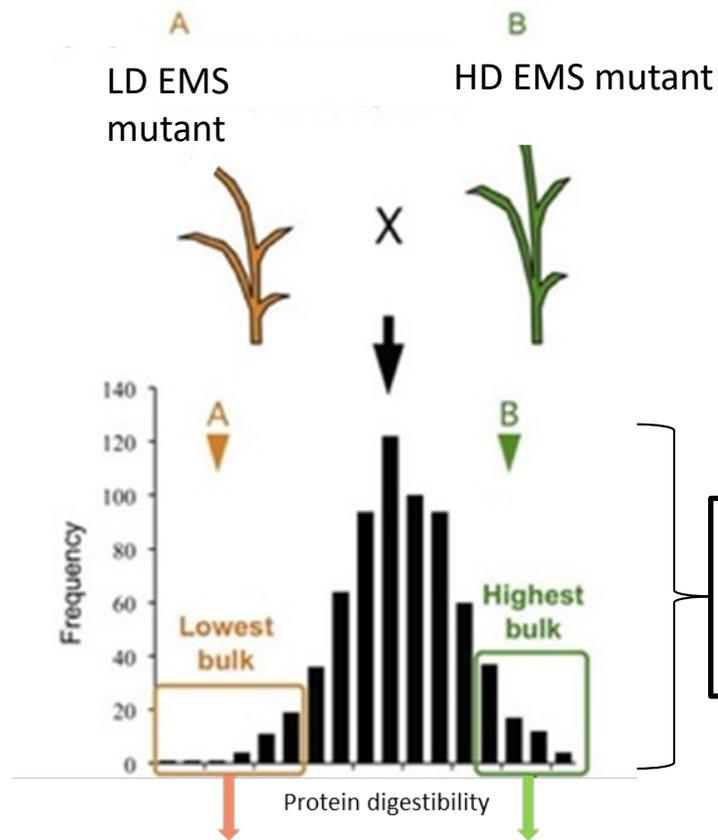
- 1.1 Develop and advance breeding populations for highly digestible protein using hl from P721Q.
- 1.2 Introgress highly digestible protein traits from EMS mutants into elite West African cultivars.
- 1.3 Work with partners on end-product testing (bread and couscous) and in animal feeding trials (poultry).
- 1.4 Share material with regional SMIL collaborators.





## 1.2 Introgress highly digestible protein traits from EMS mutants into elite West African cultivars.

# EMS Mutagenesis



Phenotype for Protein Digestibility  
506 F3 for SbEMS1613  
455 F3 for SbEMS3324

- Find recombinants
- Sanger sequenced
- Alignment

- DNA extraction (F2)
- Whole genome sequencing
- SNP calling
- Comparison High vs Low bulks SNP Frequencies

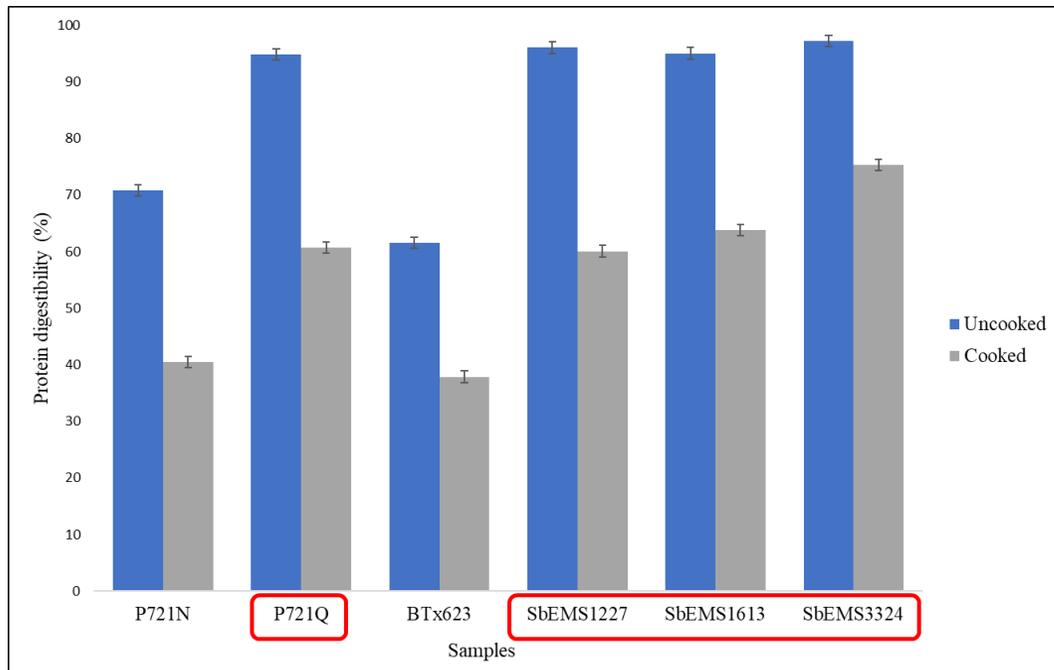
- Candidate genes identified
- PCR based primers developed and tested

Picture from Takagi et al., 2013

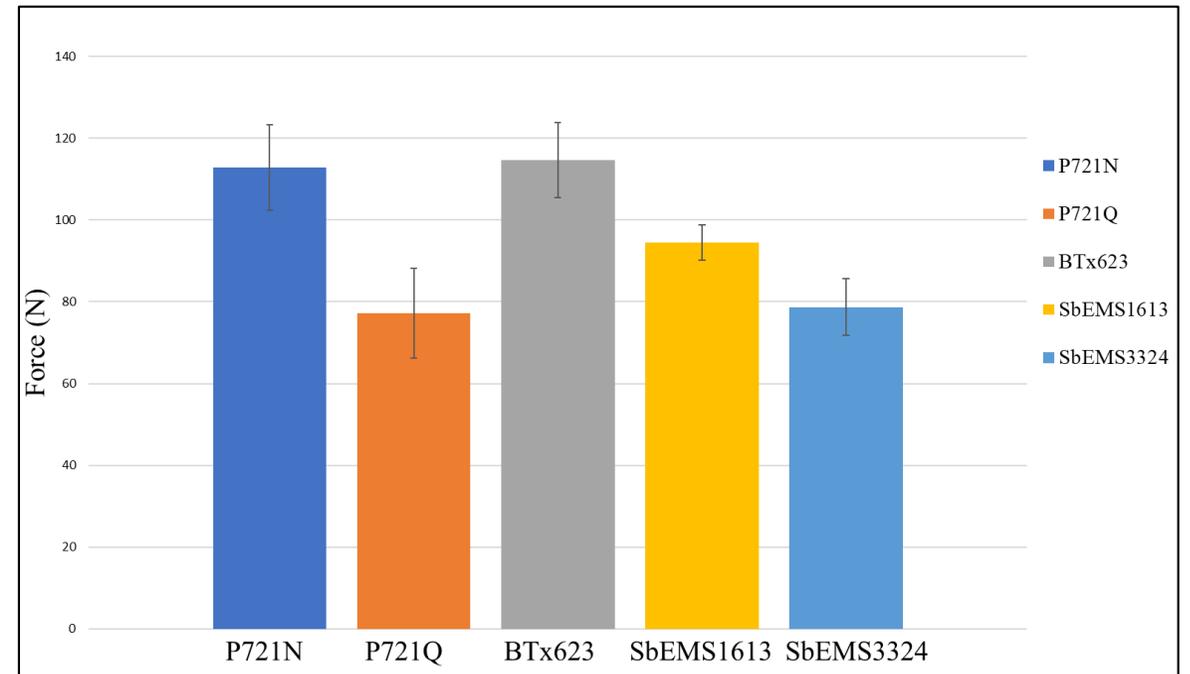
# EMS Mutagenesis

- 3 mutants with high protein digestibility were identified.

Protein digestibility



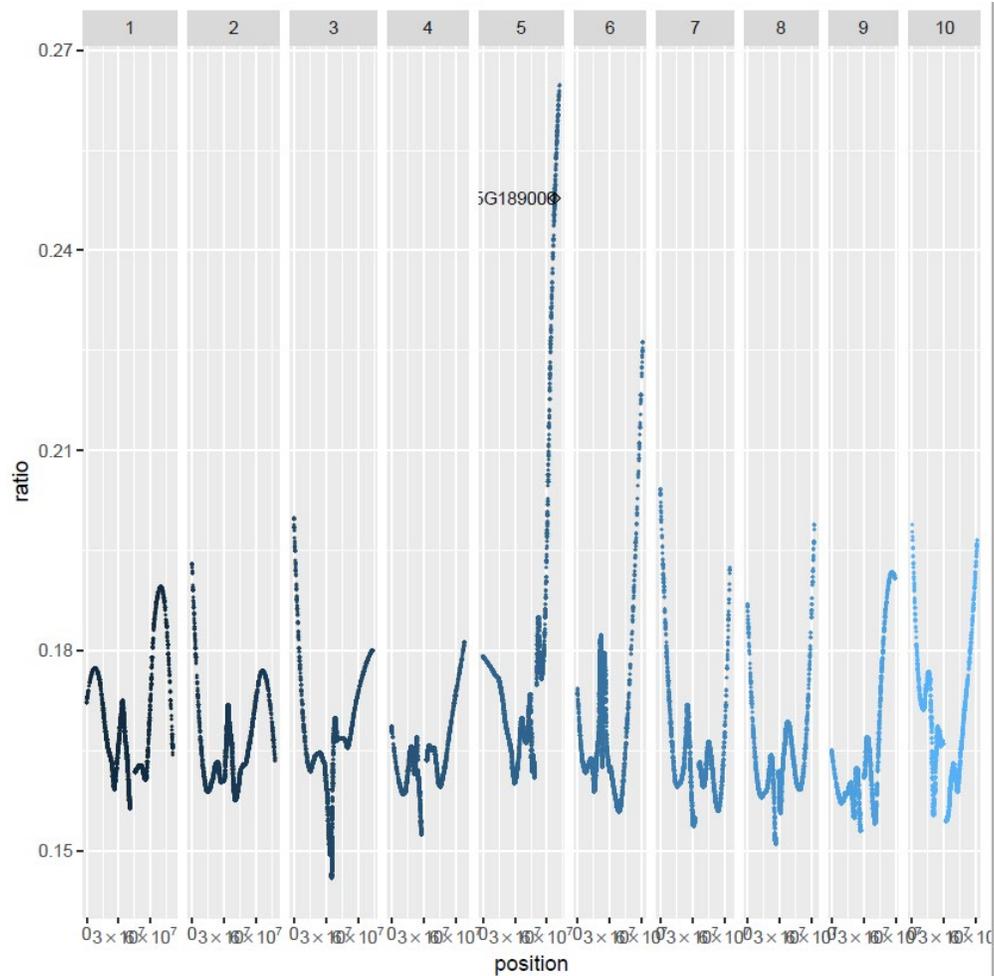
Seed hardness



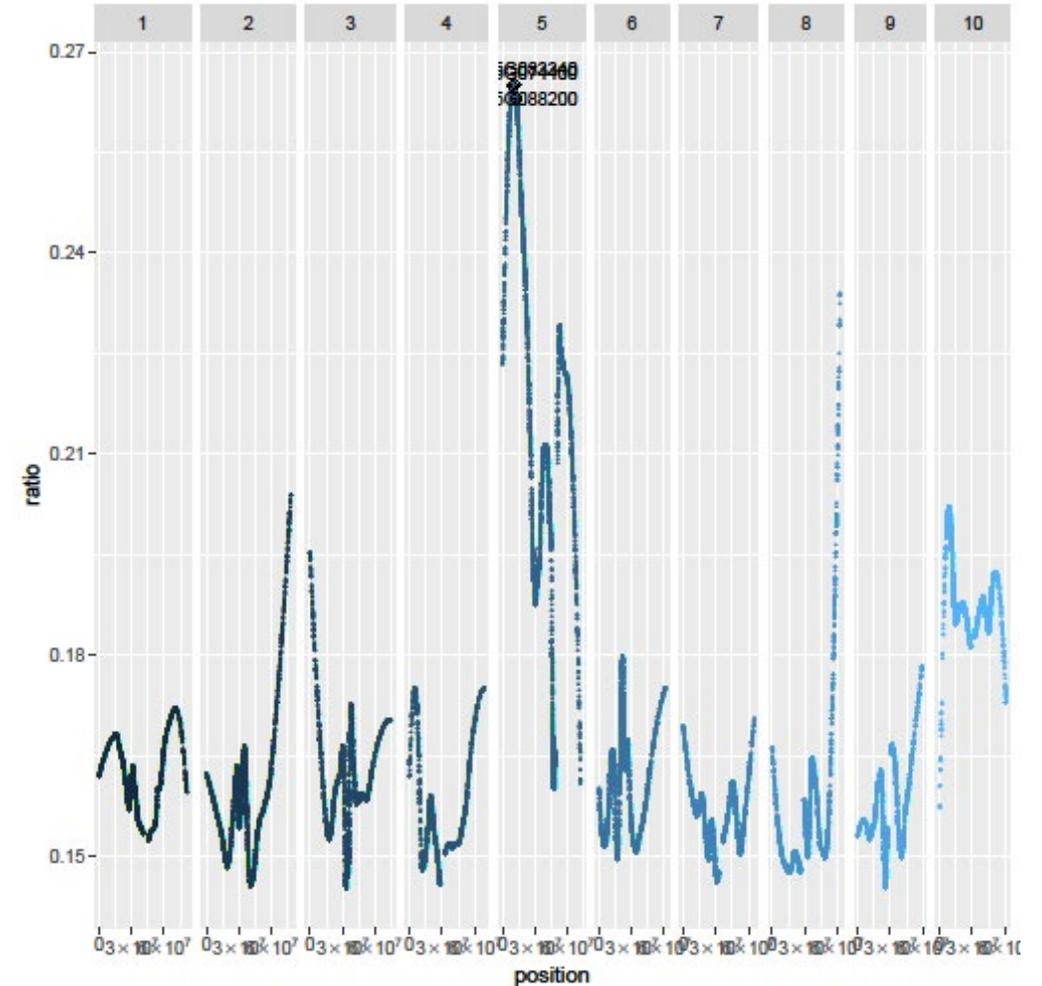
# EMS Mutagenesis

- Bulked segregant analysis

SbEMS3324



SbEMS1613





## 1.2 Introgross highly digestible protein traits from EMS mutants into elite West African cultivars.

- 1.2.1 Create genetic markers for each candidate mutation
- 1.2.2 Increase and introduce breeding populations from Purdue
- 1.2.3 Test highly digestible genotypes in multi-location field trials



## 1.2.1 Create genetic markers for candidate mutations

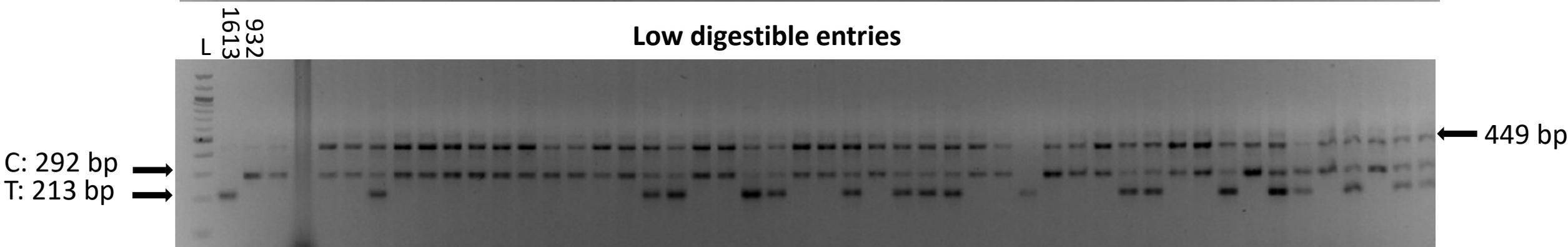
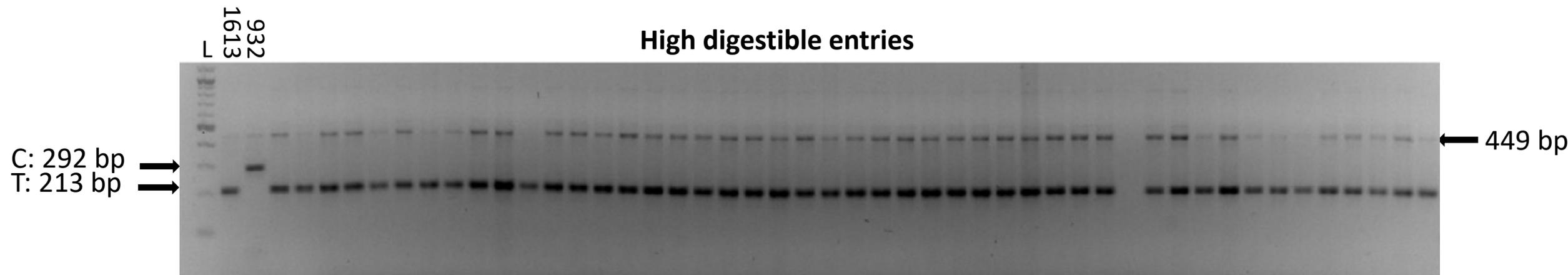
- CAPS and tetra primer markers were developed for linkage analyses in bi-parental populations.

Mutant	Chr	Mutation Effect	Candidate Genes
EMS1613	5	Splice donor variant & intron variant	SORBI_3005G074400
EMS1613	5	Missense variant	SORBI_3005G083340
EMS1613	5	Missense variant	SORBI_3005G088200
EMS3324	5	Missense variant	SORBI_3005G189000



## 1.2.1 Create genetic markers for candidate mutations

Candidate 1: Similar to Protein Disulfide Isomerase: **0 recombinants**

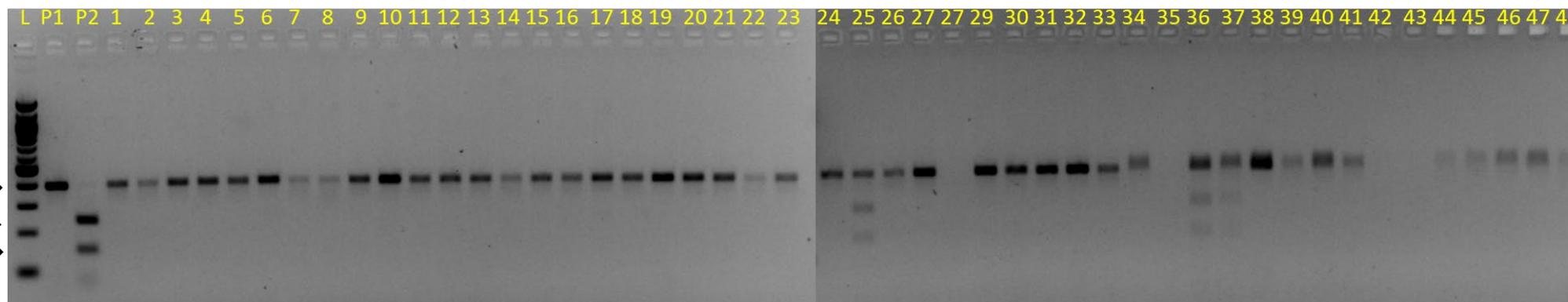




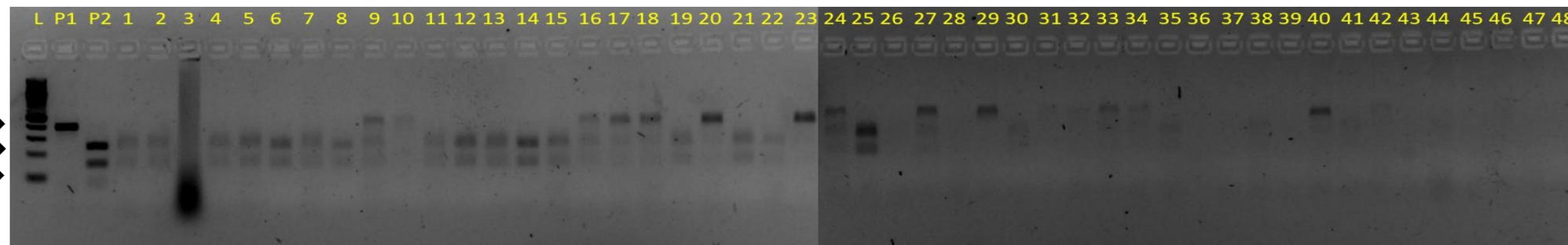
## 1.2.1 Create genetic markers for candidate mutations

Candidate 2: 26S Proteasome Regulatory Complex: **7 recombinants: 25, 36 & 37; 10, 23, 29 & 40**

High digestible samples



Low digestible samples





## 1.2.1 Create genetic markers for candidate mutations

### Analysis of the recombinants

- 7 recombinants identified and planted in a greenhouse
- A total of 70 recombinants genotyped + EMS1613 (P1) and EMS932 (P2)
- Sanger Sequencing





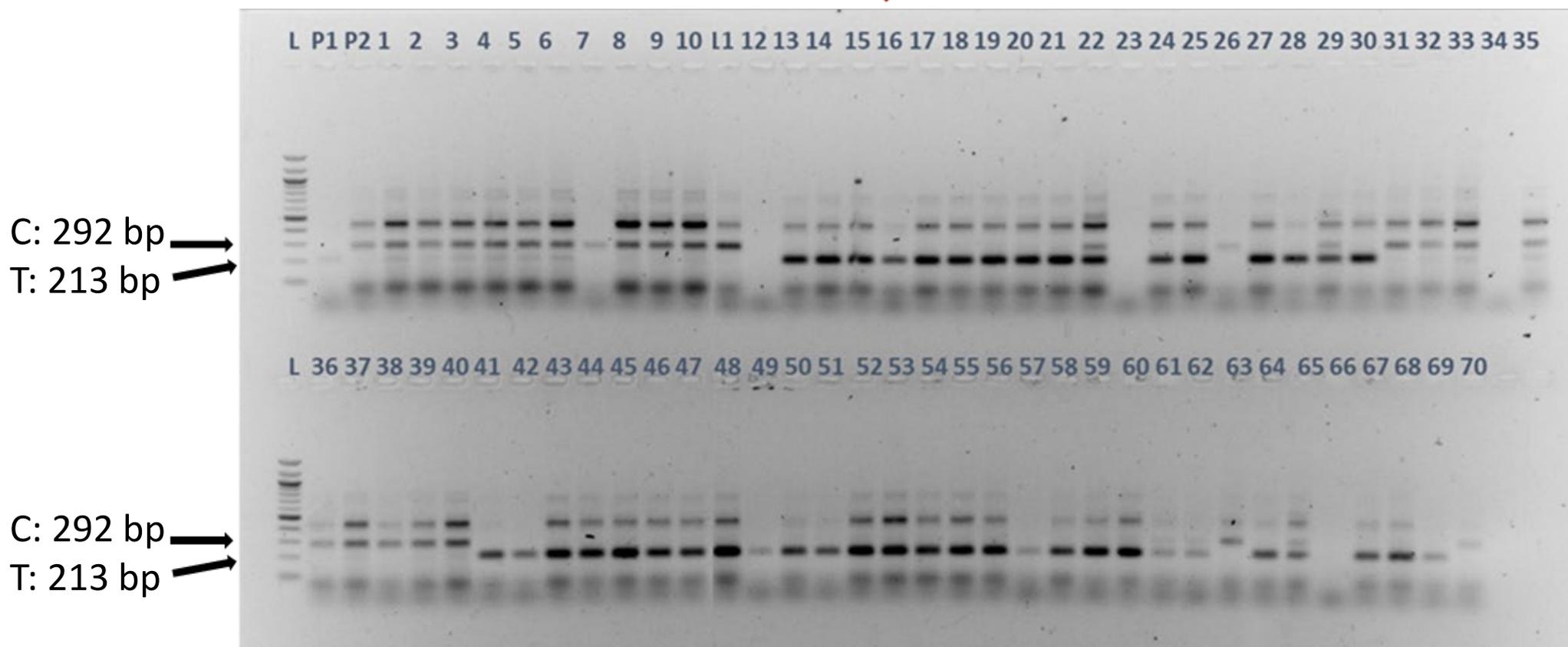






## 1.2.1 Create genetic markers for candidate mutations

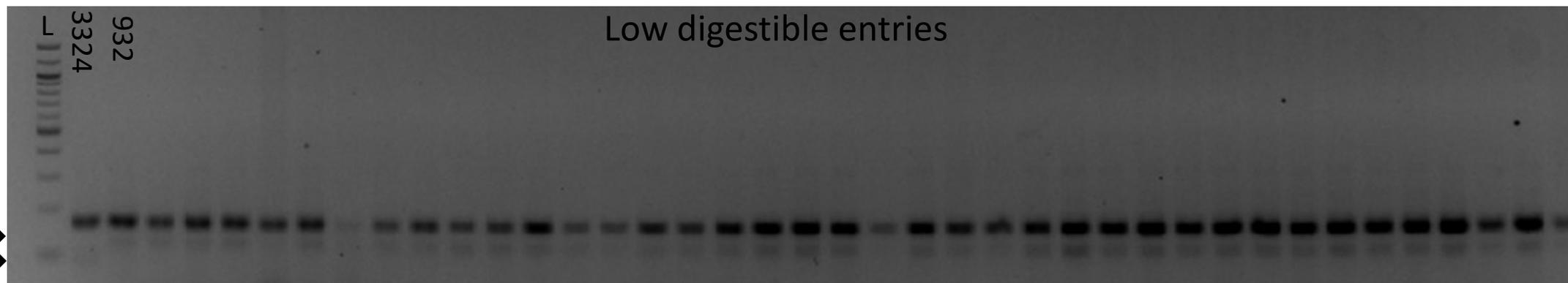
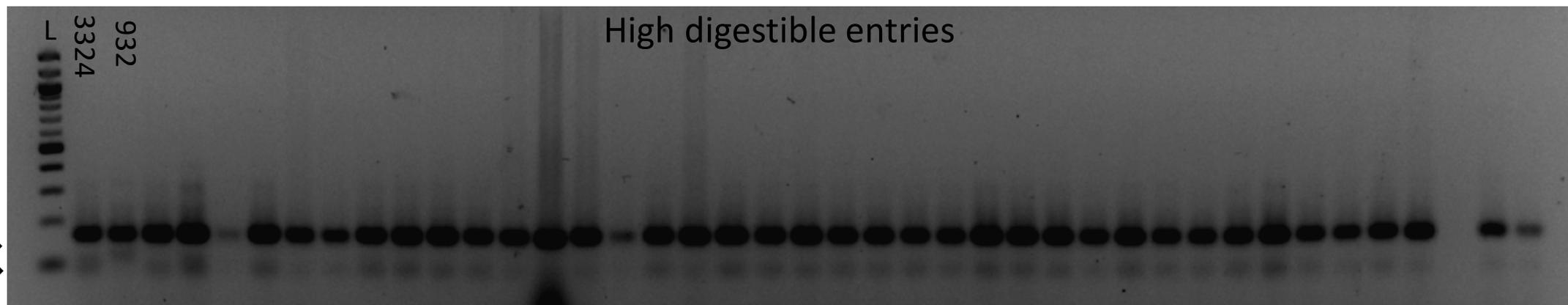
Availability of Primer Pairs - We developed markers that work too!





## 1.2.1 Create genetic markers for candidate mutations

SbEMS3324 Candidate Gene: Similar To Kafirin PSKR2 Precursor





## 1.2.2 Increase and introduce breeding populations from Purdue

- Purdue F3 breeding populations incorporating highly digestible protein traits into West African lines were introduced to Senegal
- BC3F1 and BC2F2 populations were developed during the 2021 off-season.

Population	Generations	
ISRA-S-621A × SbEMS1227	BC2F2	BC3F1
ISRA-S-621B × SbEMS1227	BC2F2	BC3F1
ISRA-S-621A × SbEMS1613	BC2F2	BC3F1
ISRA-S-621B × SbEMS1613	BC2F2	BC3F1
ISRA-S-622A × SbEMS1613	BC2F2	BC3F1
ISRA-S-622B × SbEMS1613	BC2F2	BC3F1
ISRA-S-621A × SbEMS3324	BC2F2	BC3F1
ISRA-S-621B × SbEMS3324	BC2F2	BC3F1
ISRA-S-622B × SbEMS3324	BC2F2	BC3F1



## Training and Capacity Building (2 M.Sc. students trained)

- Aminata Saou
  - Phenotypic correlation between protein digestibility and agro-morphological characteristics of sorghum [*Sorghum bicolor* (L.) Moench] BC3F7 lines in the basin.
  - Defended 8-20-2021
- Jean Noel Thiaw
  - Agro-morphological evaluation of sorghum lines [*Sorghum bicolor* (L.) Moench] with highly digestible protein in the groundnut basin of Senegal.
  - Defended 1-4-2021





## Achievements and Lessons Learned

- 3-5 lines will be proposed for registration. These combine:
  - farmer preferences (seed size, color, cycle...),
  - average yield of 2 t/ha comparable to Faourou,
  - ~66% post-cooking protein digestibility with ~20-31% increase compared to Faourou.
- Discovery of new mutations on the disulfide isomerase protein and on a kafirin that lead to improved protein digestibility.
- Two pairs of polymorphic markers were developed and will help in early selection of plants with desired trait through MAS.

**Publications:** 1 article submitted to Journal of Cereal Science.

1 poster presented at SMIL 2022 and SICNA.





## Perspectives (2022-2023)

- **Phase 1:** Populations introduced from Purdue (EMS mutants crossed to Nganda, Darou, Nguinthe and Faourou) will be advanced and used for marker validation.
- **Phase 2:** 9 sorghum lines (BC3F6) will be tested in 5 locations in 2022 rainy season and the best will be scaled up to phase 3.
- **Phase 3:**
  - Seeds of 3 to 5 sorghum lines (Faourou x P721Q) with ~20-31% more digestible proteins will be multiplied during off-season 2022.
  - Initiate release process.
  - The 2 genetic markers developed will be validated at CERAAS.



## Perspectives (2022-2023) Cont'd.

- Share seeds with partners:
  - Farmers (RESOPP)
  - Industries (SEDIMA, NMA, Mamelles Njaboot)
- Work with partners on end-product testing (porridge, bread and couscous) and in poultry feeding trials.
- Scale-up (Phases 3 and 4).



## Activity 2. Develop forage sorghum cultivars that meet the needs for increased forage production in Niger and West Africa

- 2.1 Establish the forage research program at INRAN.
- 2.2 Develop open-pollinated forage sorghum varieties.
- 2.3 Develop dual purpose grain sorghum hybrids, Sudangrass hybrids, and forage hybrids.





## 2.1.1 Fully operational seed lab

- Locality: INRAN/CERRA/NIAMEY
- Members: 1 PhD, 1 Msc, 3 Undergraduates
- Research Facilities
  - 1 dry laboratory with benches (slab) for seed processing
  - 1 office
  - 1 nursery



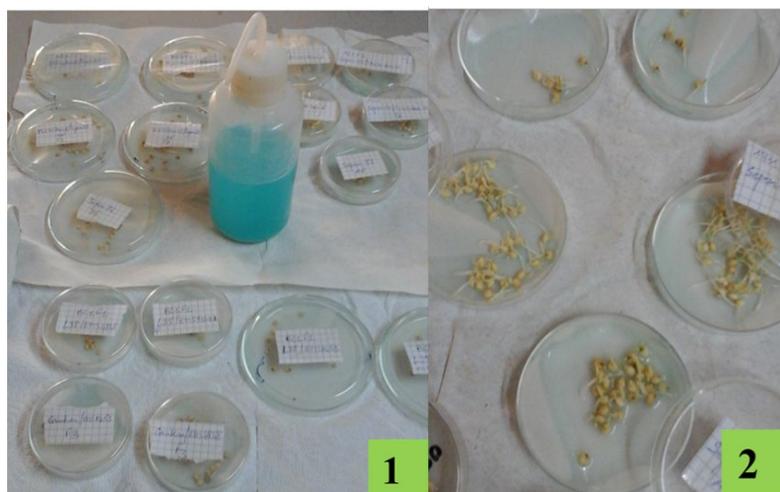


## 2.1.2 Establish field nursery sites

- 2 Nurseries (Niamey and Maradi)
  - Advance lines, increase seeds and crosses
    - OPVs
    - Forage and Sudangrass Hybrids



Adaptation of Sudangrass *bmr6*



# PATHOLOGY: Working with INRAN pathologist on stover quality



Maradi, September 2021:  
With M. Issa Karimou, Inran pathologist



Bengou, September 2021: With  
Mme Biba, Inran pathologist

# ENTOMOLOGY: Studies of susceptibility of *bmr* seed to storage insects

## Research Strategy

- **Trials Team:** breeder & entomologist
- **Site:** INRAN/NIAMEY
- **Experimental design:** CRD
- **Entries:** 8 (6 *bmr* OPVs & 2 Controls)
- **Insect:** *Tribolium castaneum* (Red flour beetle)
- **Initial infestation:** 10 adults for the bottles and 20 adults for the bags





## 2.1.3 Establish yield trial program

### Research Strategy

- Trials Team: breeder, pathologists, animal scientists & agronomist
- Sites: Kollo, Bengou, Konni and Maradi
- Experimental design: Alpha lattice
- Entries: 60 bmr OPVs, parental lines & controls



Plot-1	Rep	Site	Bloc	PLOT	Num VAR	VAR	50% FL	PH	GY (kg/ha)	DM (Kg/ha)	FSY (kg/ha)	LoG (1-5)	FoID
4056	1	KN		6	6	24							
5025	2	KN		4	5	4							
6040	3	KN		3	10	2							
4010	1	KN		2	8	57							
5067	2	KN		5	7	47							



## 2.2.1 Produce seed of 20 BC1 progenies with superior performance

- The *bmr6* and *bmr12* genes are being introgressed (BC1F6 and F7) into elite Nigerian OPVs and breeding lines (El Mota, IRAT 204, Sepon-82, MR732, L28, Grinkan, Macia, Wassa)



SHOT ON A56 Pro  
itel DUAL CAMERA



**Early Hegari/Macia//MR732 *bmr6***

Grain yield: ~2,5 t/ha

Stover yield ~8,3 t/ha

Foliar disease resistance





## 2.2.2 Conduct multi-location testing of 20 BC1 progenies with superior performance



**Group meetings**



**Meeting with farmers**



**Media**

# AGRONOMY: Optimizing stover production

**Objective:** Boost stover production

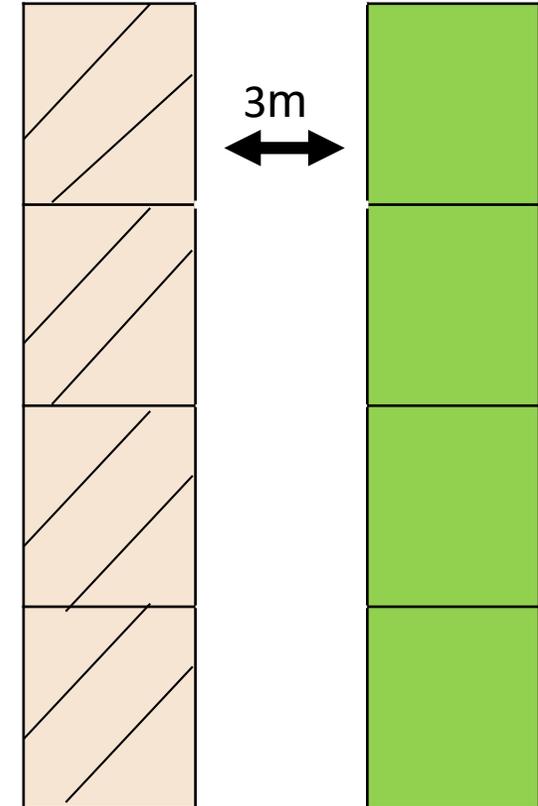
**Research Strategy:** Double cutting of biomass

- First cut: After 8 weeks of cropping
- Second cut at harvest time

**Trials Team:** Breeder, Animal scientists & Farmers

**Sites:** Maradi, Konni, Bengou and Kollo

**Experimental Design:** Simple (10m\*10m per entry)

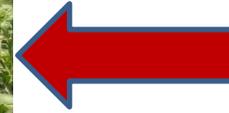


# AGRONOMY: Optimizing stover production

Plot with biomass cutting



Plot without biomass cutting



Stover samples drying under shade



# AGRONOMY: Optimizing stover production

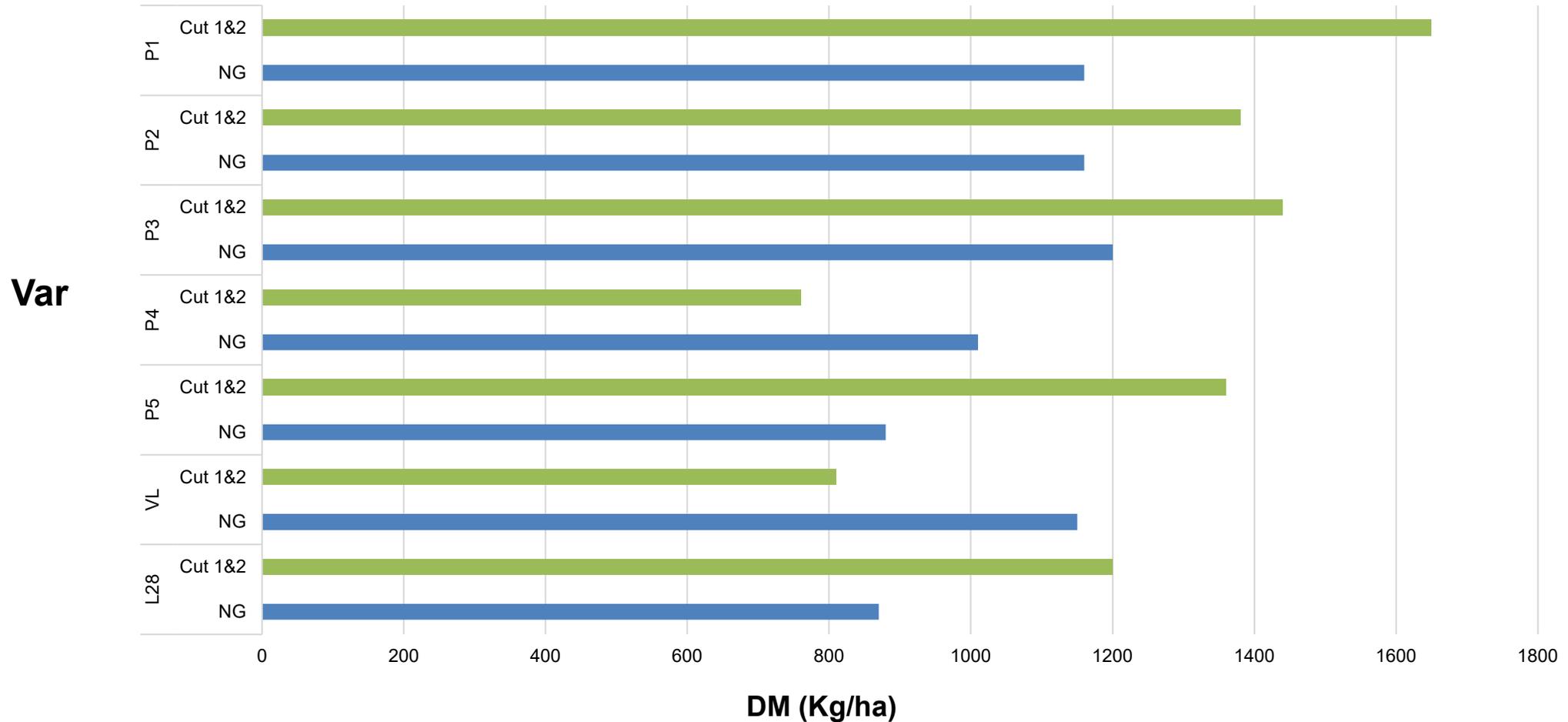
EarlyHegari/CSM-63//Wassa *bmr6*

5 weeks after  
biomass  
cutting



# AGRONOMY: Optimizing stover production

## Biomass production



**P1**=Early Hegari/Macia//Sepon82 *bmr6*; **P2**=Early Hegari/Macia//MR732 *bmr6*; **P3**=Early Hegari/Macia//Macia *bmr6*; **P4**=Early Hegari/CE-151-262-A1//CE-151-262-A1 *bmr6*; **P5**=Early Hegari/CSM 63//Wassa *bmr6*; **VL**=Local variety, **L28**=improved variety

# ANIMAL SCIENCE: Feeding Trials

**Objective:** Determine nutritional values of feeds based on new *bmr* sorghum lines

## Research Strategy:

- ❑ Duration: 42 days
- ❑ Animals: 24 goats (18-24 months; average weight  $24.39 \pm 1.11$  kg)
- ❑ Data collection: 3 times per week

**Trial Team:** Breeder, Animal scientists

**Sites:** Maradi

**Experiment Design:** CRD

- ❑ 4 replications

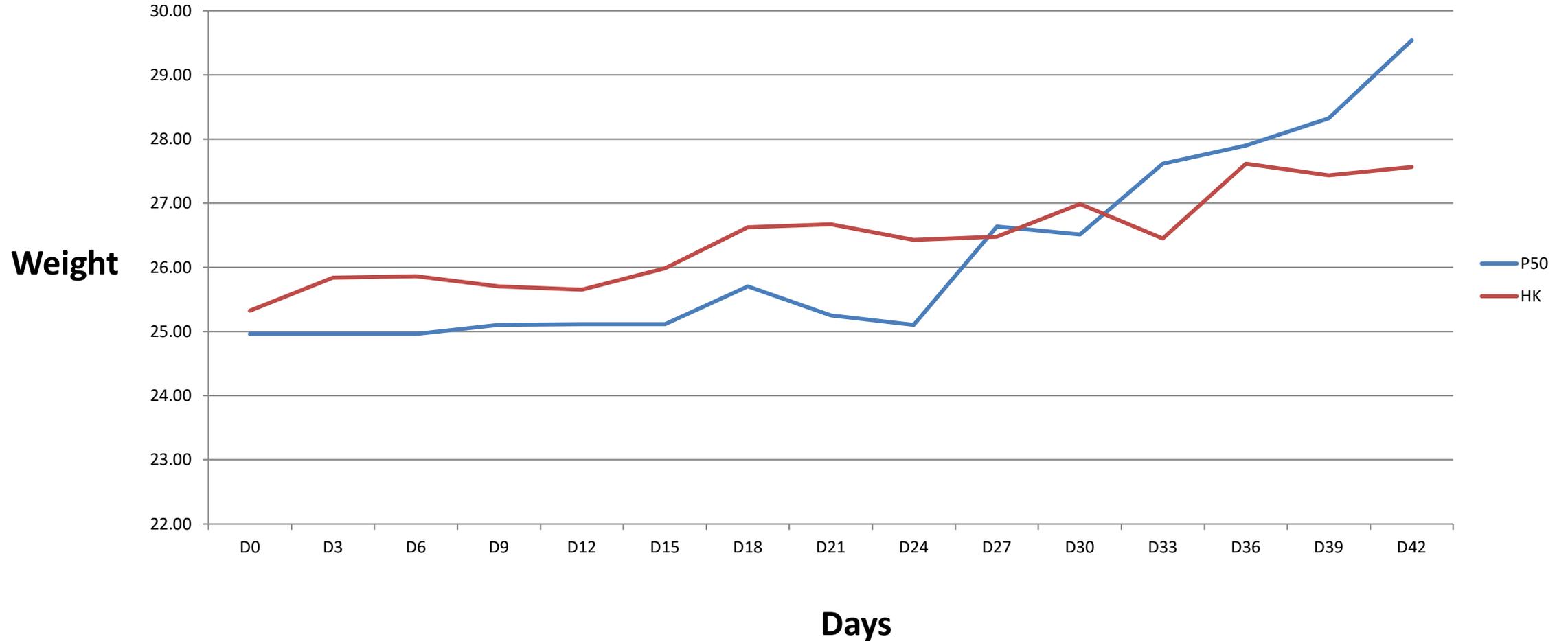


# ANIMAL SCIENCE: Feeding Trials

	Treatment					
	P50	P98	P90	HK	P35	P60
Dry Matter Intake (g/d)	861,45	716,49	886,44	<b>824,62</b>	822,89	911,82
Manure Average Quantity (g/d)	136,34	94,11	168,75	<b>144,82</b>	189,82	160,94
Initial Weight	24,96	22,55	25,69	<b>25,33</b>	24,06	23,73
Final Weight	29,54	26,10	30,36	<b>27,57</b>	28,18	27,99
Weight Gain (kg)	4,58	3,55	4,68	<b>2,24</b>	4,11	4,26
Average Daily Weight (g/d)	108,93 ✓	84,52 ✓	111,31 ✓	<b>53,37</b>	97,92 ✓	101,49 ✓
Consumption Index (kg DMI/kg gain)	7,9	8,5	8,0	<b>15,5</b>	8,4	9,0

P50=Early Hegari/Macia//Sepon82 *bmr6*; P35=Early Hegari/Macia//MR732 *bmr6*; P60=Early Hegari/Macia//Macia *bmr6*; P90=Early Hegari/CE-151-262-A1//CE-151-262-A1 *bmr6*; P98=Early Hegari/CSM//Wassa *bmr6*; HK=Local variety

# ANIMAL SCIENCE: Feeding Trials



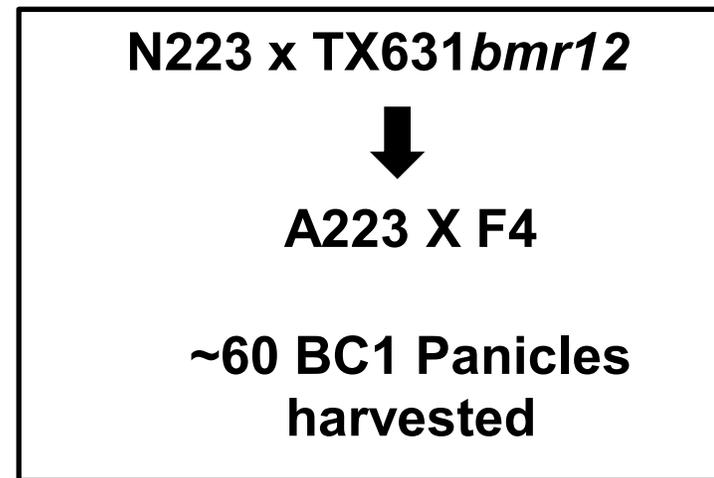
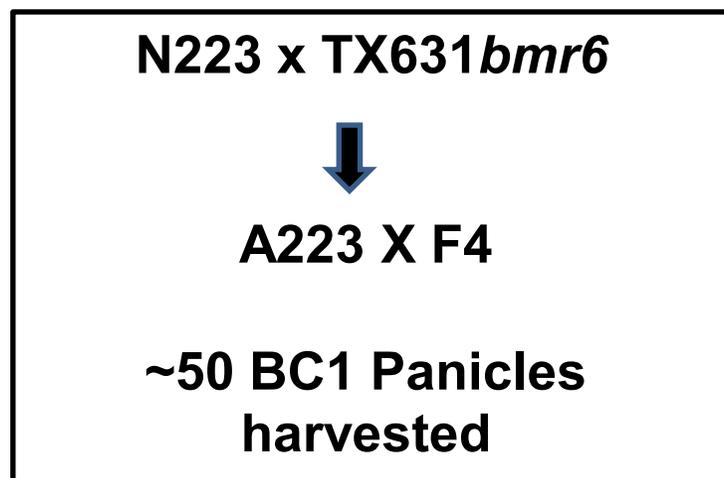
**P50 : Early Hegari/Macia//Sepon-82 *bmr6***

**HK: Hakorin Karoua (control)**



## 2.3.1 Seed parent development for future forage hybrids

- A seed parent breeding program based on Nigerien adapted germplasm was sent to INRAN to support the hybrid development efforts.
- A/B pairs are being advanced in the breeding program.





## 2.3.2 Pollinator parent development for future forage hybrids

- A collection of forage pollinator breeding lines were developed and sent to INRAN to support the hybrid development efforts.
  - Caudatums exhibited good adaptation and potential
  - One sudangrass pollinator also exhibited good adaptation
- *Brown-midrib* conversions of MR732 and Excel S235 are being developed at Purdue and distributed for hybrid development in Niger.





## Training and Capacity Building (8 students)

Lycée agricole de Tera: 3

- Sorghum breeding populations development
- Sorghum pure line seeds production
- Sorghum F1s identification using morphological traits

IPDR/Kollo: 2

- Optimizing stover production
- Variety selection

University of Tillaberi: 1 and National service: 2

- seed preparation, trial conduction, nursery work





## Training and Capacity Building

### 3 fact sheets produced

- **Caracteristiques morphologiques des sorgho *bmr* (INRAN)**
- **Processus de régénération de plantules de sorgho obtenues à partir de l'émascation manuelle des fleurs (INRAN).**
- ***Bmr* sorghum cultivation in Niger & west Africa (iREACH)**





## Plans for 2022

- Phenotyping trials will be conducted in 2022 in Tillabery, Kollo, Gaya, Konni and Maradi.
- Participatory varietal selection with farmers to select best performing lines to be released. We are targeting 200 farmers for fields visit
- A good volume of data will be collected for *bmr* sorghum varieties registration (we are targeting to register 7 new varieties)



## Achievements and Lessons Learned

- OPVs with good adaptation and forage quality attributes were identified in multi-location trials.
- Farmers exhibited preference for many of the *brown-midrib* accessions over the local varieties.
- The animal feeding test showed the great potential of *bmr* stover over farmers 'normal' variety



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