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

2021-22 Annual Progress and Plans

SMIL Project

Enabling Marker Assisted Selection for Sorghum Disease Resistance in Senegal and Niger

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Collaborative Research on Sorghum and Millet
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TAMU- Student Arrives

MAY 25, 2021

Coumba Fall, arrived from Senegal after well more than a year delay due to COVID

On May 17, Coumba was forced to return to Senegal from Paris despite a USAID authorized VISA

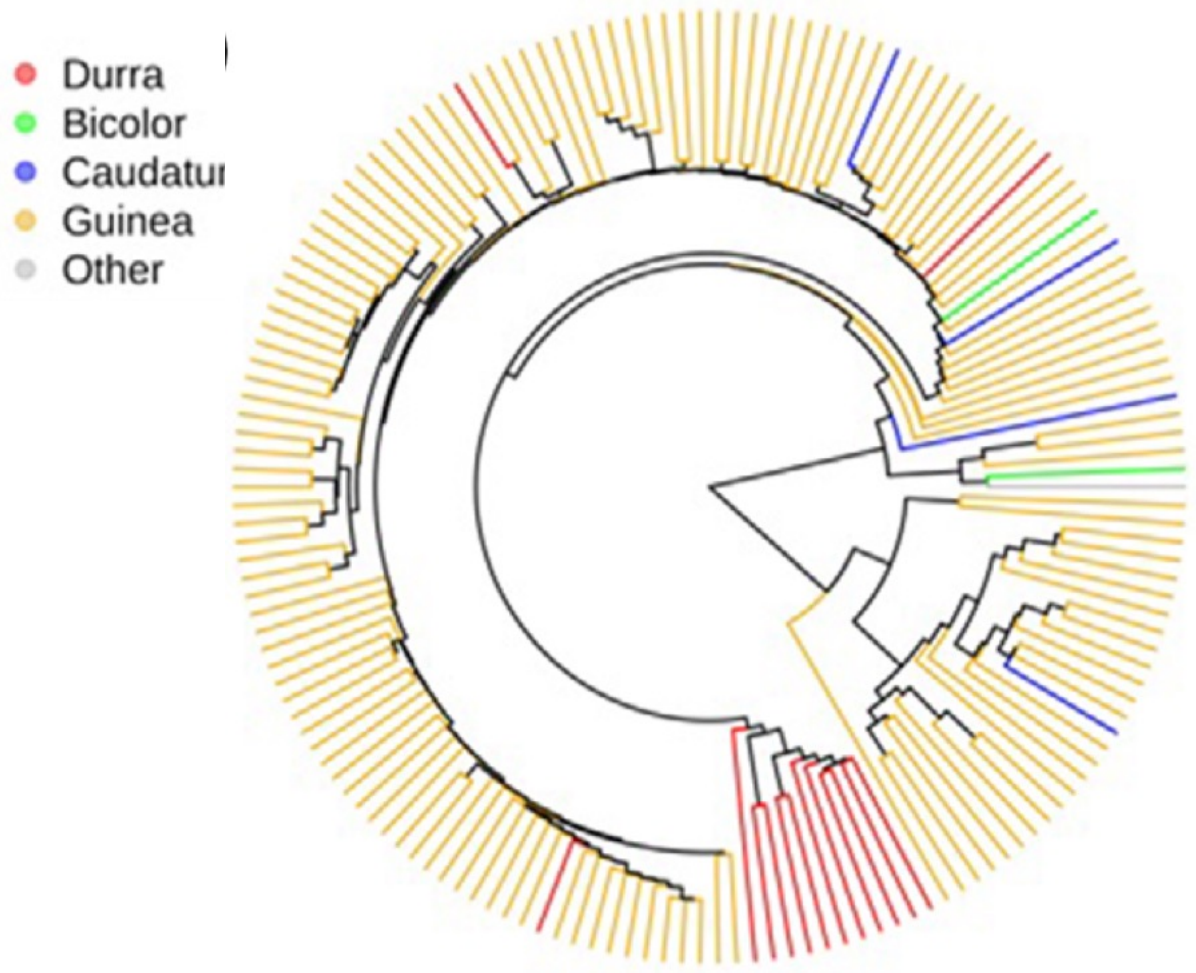
We learned her MS degree does not count toward required PhD hours at TAMU

Advisory committee established: includes Louis Prom, Tom Isakeit, a sorghum extension expert, and Endang Septiningsih, a crop biotechnologist in Soil & Crop Sciences

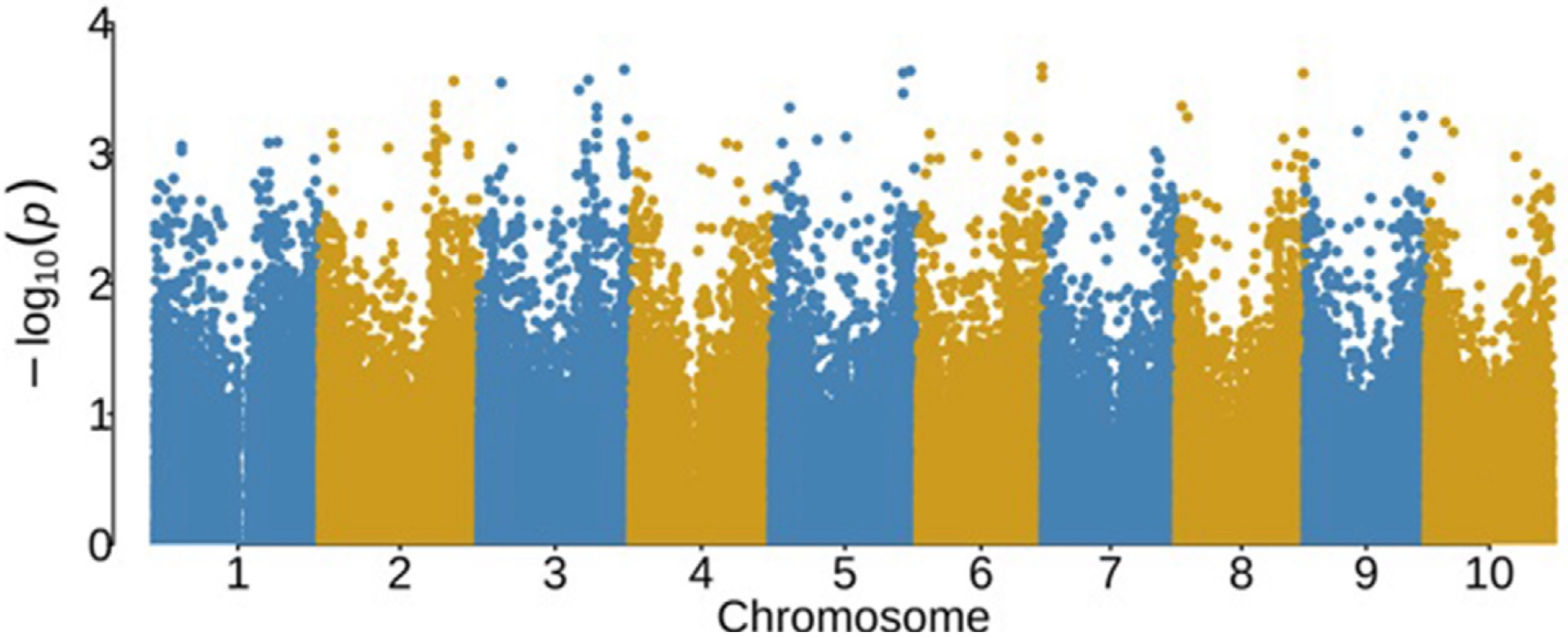
So far, all A's

In the meantime

- Identified 163 sorghum accessions from Senegal in the USDA sorghum germplasm collection that Geoff Morris already had sequenced.
- Grew in greenhouse and tested for response to inoculation with a mix of 8 local (Texas) isolates of *Colletotrichum sublineola*, which causes anthracnose. (3 reps)
- 88 lines scored as resistant
- 75 lines scored as susceptible (pathogen reproduction occurred)



GWAS Based on almost 200,000 SNPs



Genes Nearest Top 8 SNPs

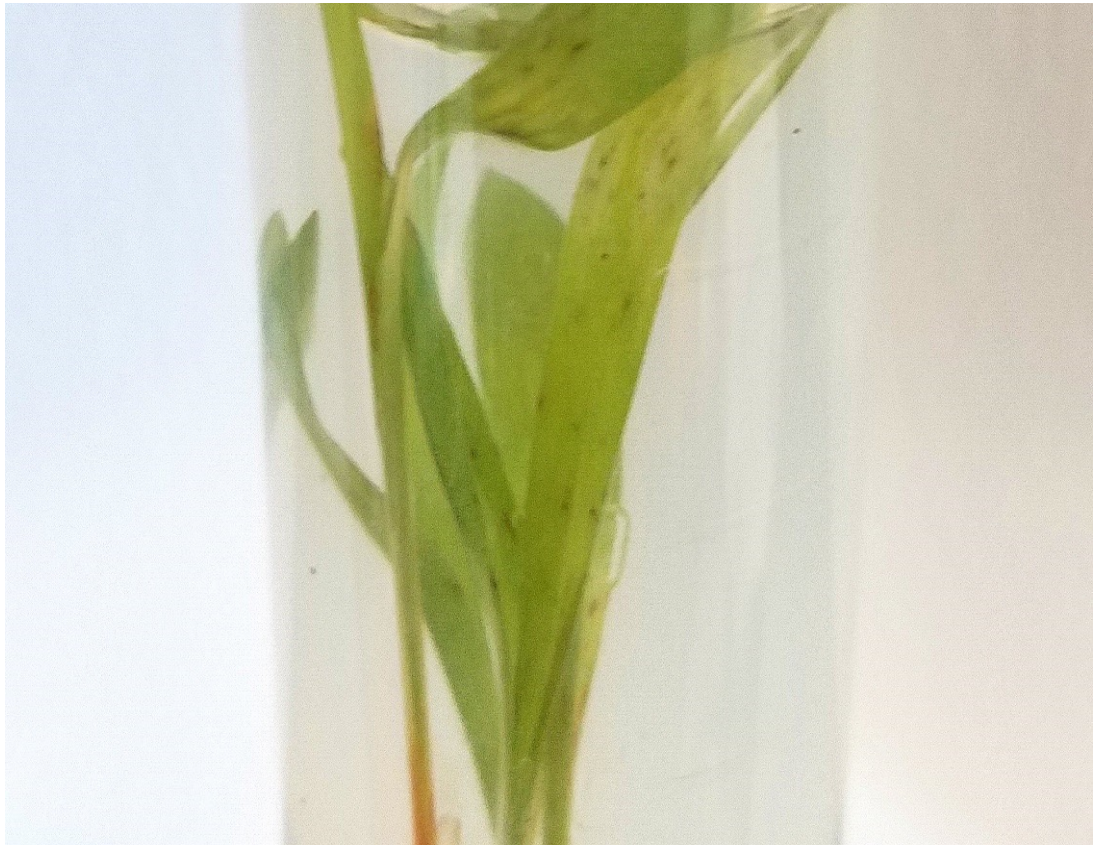
TABLE 2 Annotated genes nearest to the most significant SNPs associated with anthracnose. The distance in base pairs to the nearest genes and *p* value are listed

Chr.	Location	Candidate gene	Associated annotation	Base pairs	*P* value
6	60609133	Sobic.006G274866	Leucine Rich Repeat // Protein tyrosine kinase // Leucine rich repeat N-terminal domain	4,656	.000219
3	70974745	Sobic.003G401200	Selenium binding protein	15	.000229
5	67841876	Sobic.005G194700	Zinc finger, C3HC4 type family protein	0	.000234
5	64406687	Sobic.005G166700	Sulfotransferase family	5,538	.000243
8	61651261	Sobic.008G183100	Protein of unknown function// Domain of unknown function	0	.000244
3	53329242	Sobic.003G203500	Cytosolic aldehyde dehydrogenase RF2C Aldehyde dehydrogenase family	1,983	.000274
2	65282291	Sobic.002G268900	Single strand DNA repair-like protein	0	.00028
3	10745341	Sobic.003G118600	F-box domain	10,377	.000288

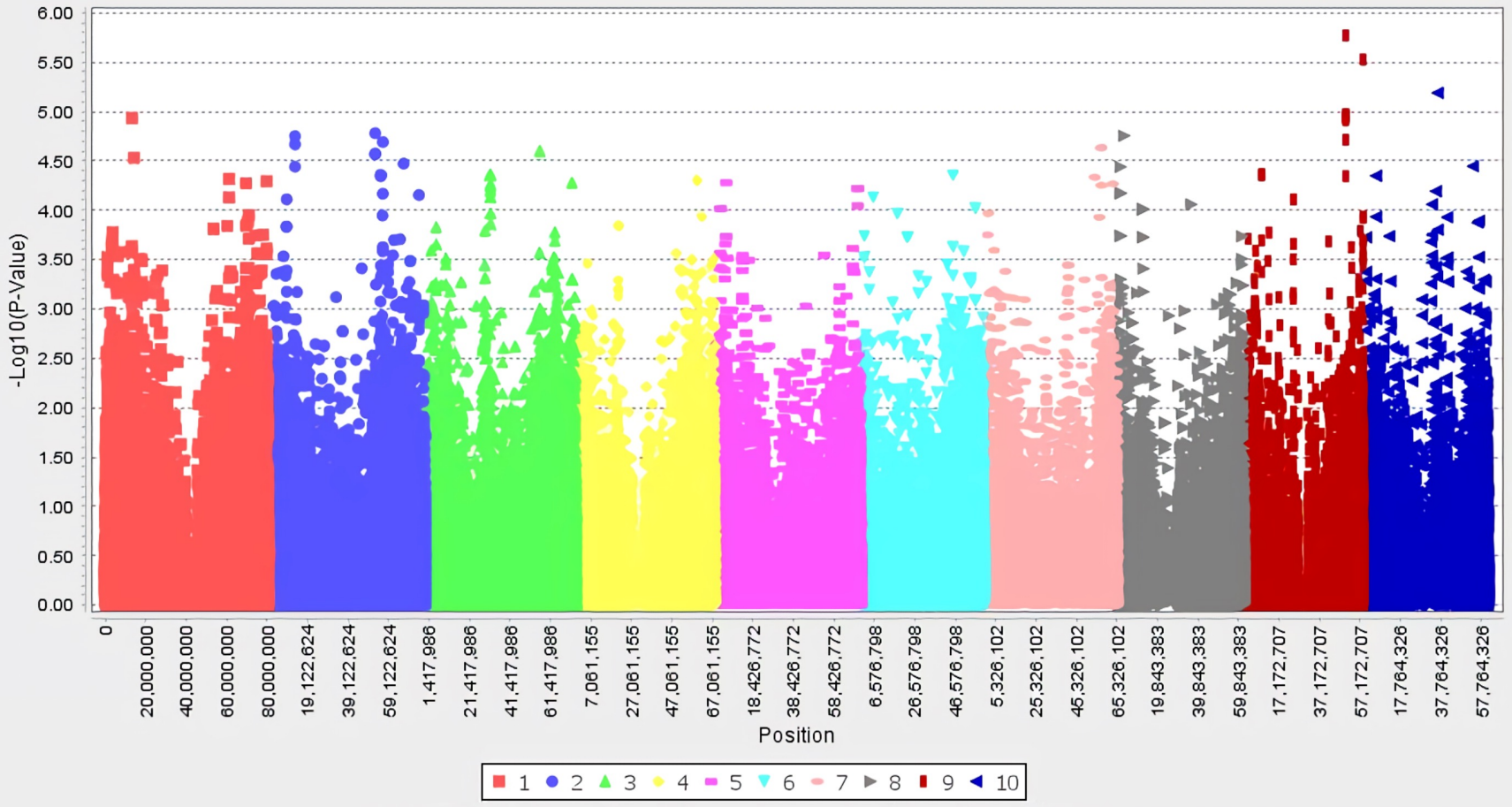
Note. Chr., chromosome; SNP, single-nucleotide polymorphism.

Tests for headsmut

- The same cultivars are being used to test for response to headsmut caused by *Sporisorium reiliana*. Rather than wait for all plants to head out Ezekiel and Coumba are also testing a procedure that can use immature leaves, with responses as shown at the right. Confirmation will rely on results in greenhouse tests.



GWAS based on response to headsmut pathotype 5



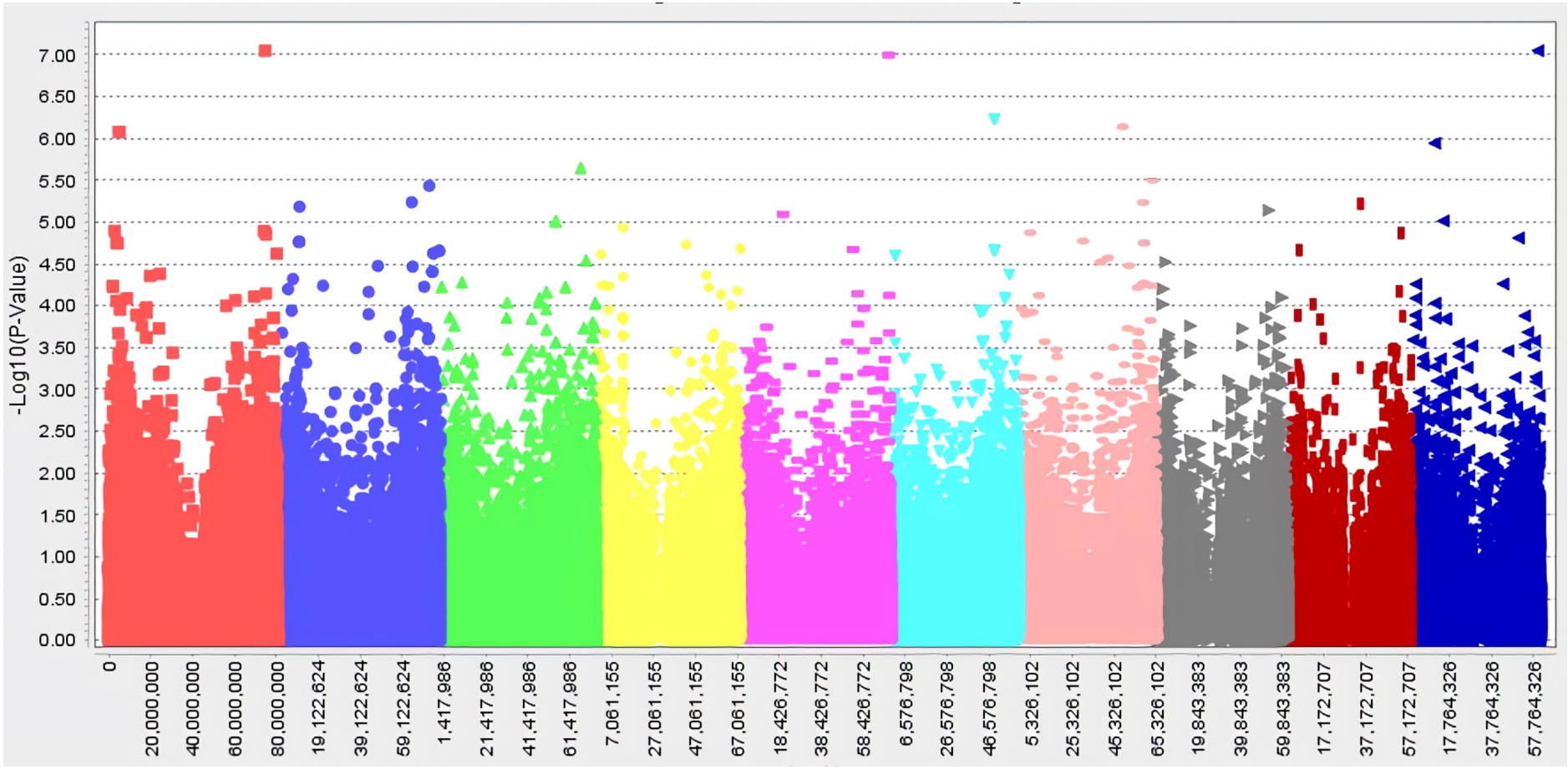
Nearest Genes

The top 'hit' on chromosome 9 is related to starch formation in plastids. In Arabidopsis, reduced starch levels have been associated with impairment of resistance to penetation by *C. higginsianum*[1]. Rice AAA-ATPase has a demonstrated role in blast resistance[2] while an ATP-binding cassette transporter regulates pathogen resistance by affection jasmonate biosynthesis[3]. Consequently, it is easy to imagine these genes could affect host-pathogen interacrtions in detached, submerged sorghum leaves.

Chr	Location	Candidate Gene	Associated Annotation	Base pairs	*P*-value
9	50150940 and 6 more within 3200 SNPs among top 20 SNPs	Sobic.009G144200	Similar to Glycogenin-like protein	5801	0.0000017
9	58701294	Sobic.009G252400	AAA-type ATPase	0	0.000003
10	36147029	No nearby annotated gene			0.0000065
1	13312936	Sobic.001G161700	Vesicle-fusing ATPase	137	0.000012
2	52606601	Sobic.002G168200	ATP-binding cassette transporter	953	0.000017
8	3301850	Sobic.008G035800	Similar to Glycosyltransferase 3	0	0.000018

1. (https://doi.org/10.1093/jxb/erw434) 2. https://doi.org/10.3390/ijms21041443
3. https://doi.org/10.1016/j.plantsci.2020.110582

GWAS based on timing of spot appearance to *S. reiliana* pathotype 5



Nearest Genes

G3PD has a critical role in abiotic stress recovery in wheat so it's appearance on the list may result from using submerged leaves for the test. However, the list again includes an LRR protein, which is a common feature of cloned disease resistance genes and Zinc and ring finger domains suggest roles as transcription factors involved in signal transduction pathways, whether for disease, development of stress.

Chr	Location	Candidate Gene	Associated Annotation	Base pairs	*P*-value
10	59892365	Sobic.010G262433	Glyceraldehyde 3-phosphate dehydrogenase	1523	0.000000089
1	74613814	Sobic.010G262433 but closer to Sobic.001G474001	Weakly similar to Pumilio-family RNA binding repeat containing protein No annotation, but EGF domain containing protein	3958 or 177	0.00000009
5	70650432	Sobic.005G219900	Leucine-rich repeat (LRR) containing protein	0	0.0000001
6	49269621	Sobic.006G127700	Ring finger domain (zf-RING_2)	489	0.00000059
7	49330249	No nearby annotated gene			0.00000073
1	4960561	Sobic.001G065500	Zinc finger, C2H2	0	0.00000084

Lines from 2019 collection trip

- Seeds for 60 cultivars from Senegal and 60 from Niger were grown in D. Proms quarantine greenhouse as required by APHIS. Seed collected from each line was expanded in Puerto Rico. Sets of seed packets have been prepared for disease analysis in Texas, Niger and Senegal.

- DNA was extracted from leaf samples of each cultivar. Initial quality tests showed that many needed to be re-extracted and/or repurified (Coumba)

- Whole Genome Sequencing Data have now been obtained: reads/sample ranged from 1,563,256 to 7,784,865

Dried leaf samples from Niger

- 50 samples provided
- DNA extracted from each sample
  - Discolored from being in alcohol
  - Coumba found a protocol for removing pigment
  - Most samples had DNA fragments too small for standard sequencing
  - 23 samples are at the Texas Agrilife sequencing center

Challenges

- 1. non-degraded DNA samples
- 2. getting extraction kits & equipment to Niger and Senegal
- 3. Planned disease follow-up survey may require virtual component
- 4. Disease tests in all locations among seeds provided following the 2019 survey

Deliverables and Activities -Next 18 months

- Receive DNA or samples from R by S segregating crosses;
- Use sequences to identify SNPs associated with host response
- Map SNPS to the most recent sorghum genome sequence
- Develop allele specific PCR primers or
- Follow-up survey of diseases

- At least two crosses per country
- Any disease(s) for which crosses are available
- Make DNA extraction possible in both Niger and Senegal
- GWAS on anthracnose and head smut response on Senegal & Niger accessions from survey

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This study is made possible through funding by the Feed the Future Innovation Lab for Collaborative Research on Sorghum and Millet through grants from American People provided to the United States Agency for International Development (USAID) under cooperative agreement number AID-OAA-A-13-00047. The contents are the sole responsibility of the authors and do not necessarily reflect the views of USAID or the US Government.

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SENEGAL

Objectives 2021-2022

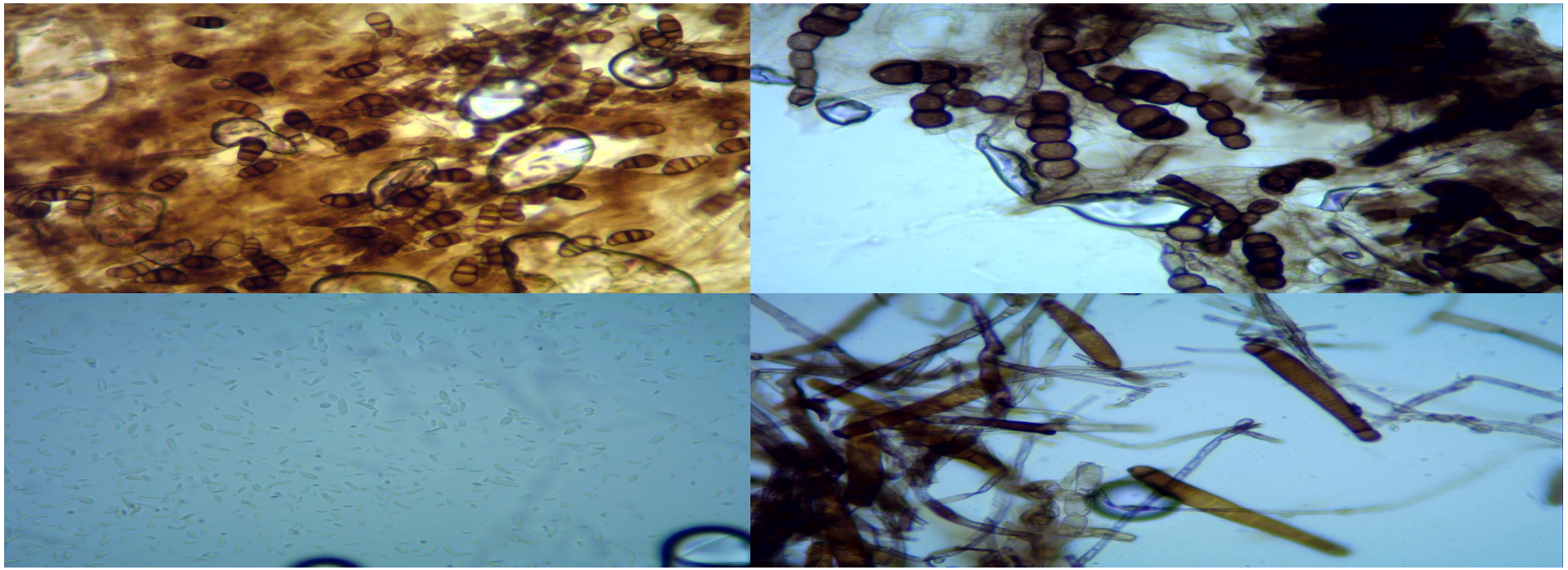
- 1-Measures of pathogen diversity
- 2-Species and race identification
- 3-Make crosses to combinations of resistance
- 4- Screening in hot spots for specific diseases under field conditions

Results 2021-2022

Exserohilum spou Helminthosporium sp

1.pathogen diversity in grain mold

- The most common fungi we actually have on our samples are: *Curvularia lunata (51,80 %), Fusarium sp. (9,18 %°, Alternaria sp (33,77%) Helminthosporium sp (4,26 %), Aspergillus sp. (0,98 %) .*



2-genetic diversity of anthracnose isolates

- DNA extraction of the anthracnose isolates were not successfull due to time to have a lab for analysis and delay for equipment purchasing.

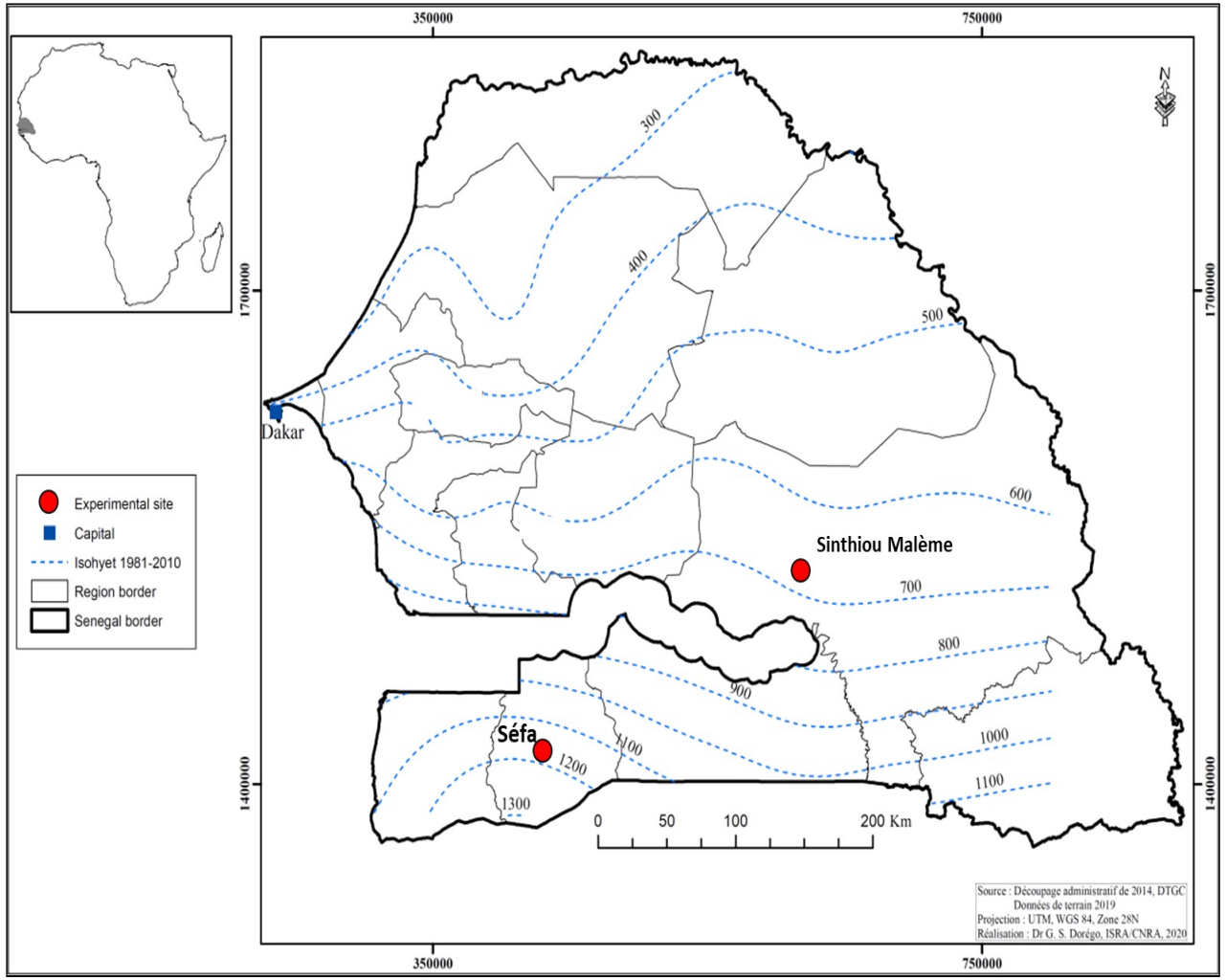
- We are now preparing spores in the lab to send them to TAMU.

3-Evaluation of populations 621A and 621 B for their resistance to anthracnose and grain mold

- 36 out of 182 lines of 621A tested in 3 locations (Bambey, Kolda and Sinthiou) were resistant to anthracnose (severity 0-2 % max) and only 01 were resistant to grain mold (severity 0 %)

- 15 lines out of 174 lines of 621B tested in 3 locations were resistant to anthracnose (severity 0 %) and any line were resistant to grain mold at the same time in the 3 locations.

Evaluation of a new collection of sorghum for grain mold, anthracnose and agronomic traits



- **256 accessions of sorghum were** evaluated at **Sinthiou Malème** and **Séfa** (ISRA stations) during rainy season.

Rainfall pattern

- *Sinthiou Maleme : 600 – 800mm*
- *Sefa : 900 – 1300mm*

Evaluation of a new collection of sorghum for grain mold, anthracnose and agronomic traits

Among 256 accessions of sorghum:

- 54 accessions were high yielding (≥2t/ha);

- *25 accessions were high yielding and grain mold resistance;*

Evaluation of a new collection of sorghum for grain mold, anthracnose and agronomic traits

- *04 accessions were high yielding and anthracnose resistance;*

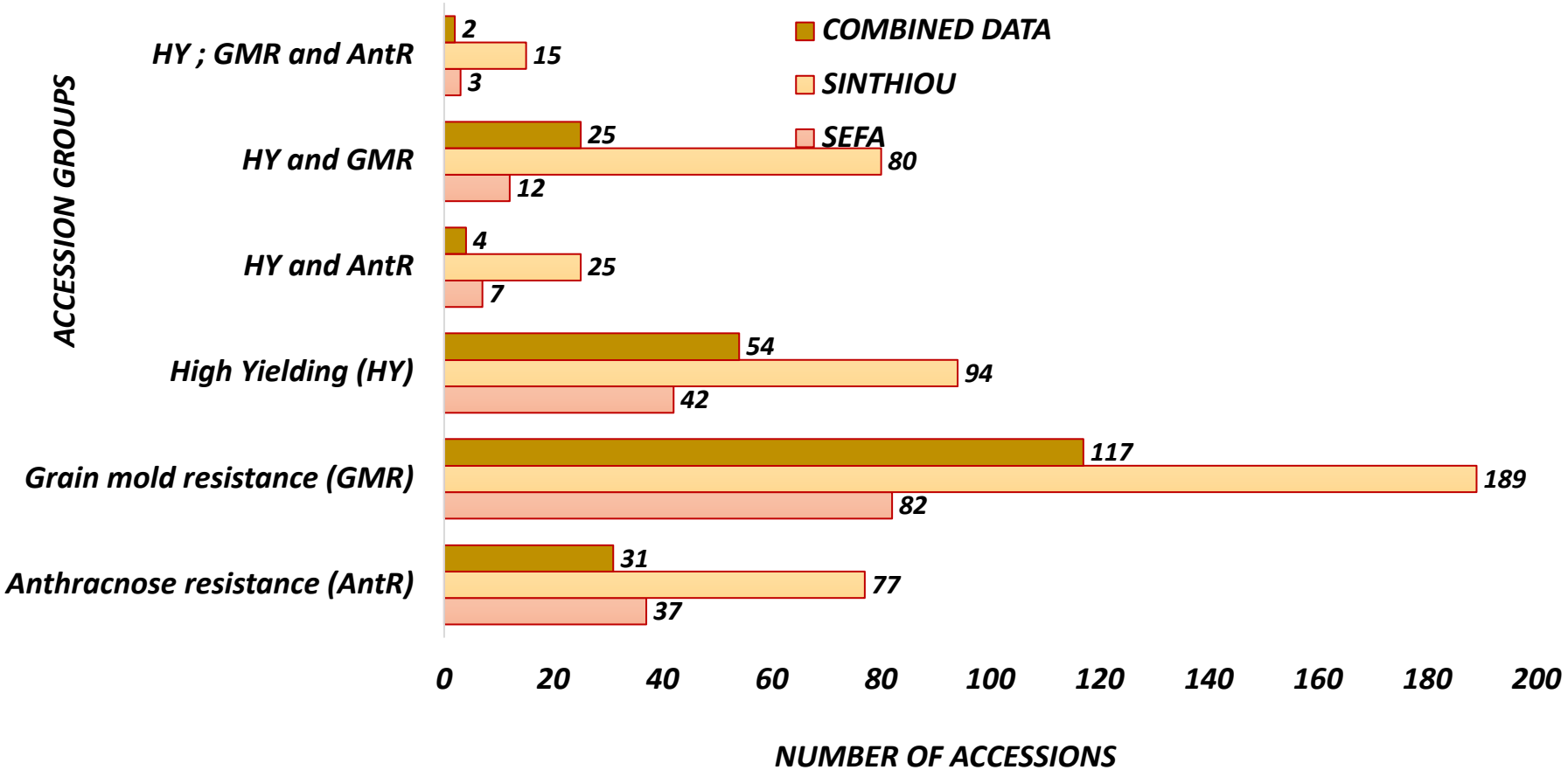
Accessions	yield	ScAnthr
CTS237	2064,2	2
CAB086	2259,4	2
CAB065	2254,5	2
CAB015	2297,6	2

- 02 accessions were high yielding, grain mold and Anthracnose resistant (see next slide).

Accessions	Yield	ScMMP
CAB086	2259,4	2
CAB065	2254,5	2

Evaluation of a new collection of sorghum for grain mold, anthracnose and agronomic traits

- **Combined data** (Sinthiou Maleme and Sefa)



Evaluation in hot spots at Tambacounda and Sefa

- 82 cultivars including the differentials lines, the ones we collected during survey and some from the national collection were evaluated in 3 hots spots: Kabendou, Missirah and Taliboulou.

- In this table, we have cultivars with less than 10 % anthracnose severity and their severity to grain mold.

Entry	Antracnose severity	Grain mold severity
Sorvato 1	5,47426	49,33
CE151-262	5,49764	3,45
ICSV88032	6,12222	94,28
CE196-7-2-1	6,13224	41,43
CE180-33	6,4295	82,4
MACIA	6,4295	93,9
CSP62	7,07913	44,75
CSP71	7,07913	87,21
CE145-66	7,07913	88,98
F2-20	7,07913	47,22
Golobé	7,09082	93,9
Grinkan	7,09082	87,21
Nguinthe	7,09082	47,22
SURENO	7,09082	7,23
CSP68	7,09917	83,41
CSP164	7,09917	39,29
CSP182	7,09917	7,23
CSP 79	7,1142	3,45
CSP85	7,1142	67,57
Payenne	7,1142	92,56
PI570726	7,1142	67,57
Darou	7,2979	65,9
Faourou	7,7488	47,22
CSP177	8,03103	11,022

Evaluation in hot spots at Tambacounda and Sefa

- From the referentials, only ICSV88032 and PI570726 had less than 10 % severity;

- All the improved varieties had less than 10% anthracnose severity except Nganda (21 %).

- For grain mold, the lowest severity are from 01 cultivar we collected during survey **CSP 79** and 01 from the new sorghum collection **CE151-262** (3,45 % of severity for both of them).

Evaluation of Anthracnose Differentials at ISRA Stations (Bambey, Roff and Sefa)

For grain mold

18 anthracnose differentials evaluated for grain mold, anthracnose and yield during the 2021 rainy season.

09 varieties showed good tolerance to grain mold at Bambey and Roff during the rainy season 2021 (score 1-3).

Variety	Grain Mold Score	
	Bambey-2021	ROFF-2021
PI570726	***1,0***	***3,3***
IC9V88032	***1,0***	***3,3***
IS18760	***1,3***	***1,7***
PI570841	***1,0***	***1,7***
THEIS	***1,7***	***2,3***
BVANDES	***1,1***	***2,0***
BTX623	***3,3***	***3,0***
PI569979	***1,0***	***3,3***
SC748-5	***1,8***	***3,7***

Evaluation of Anthracnose Differentials at ISRA Stations (Bambey, Roff and Sefa)

For anthracnose

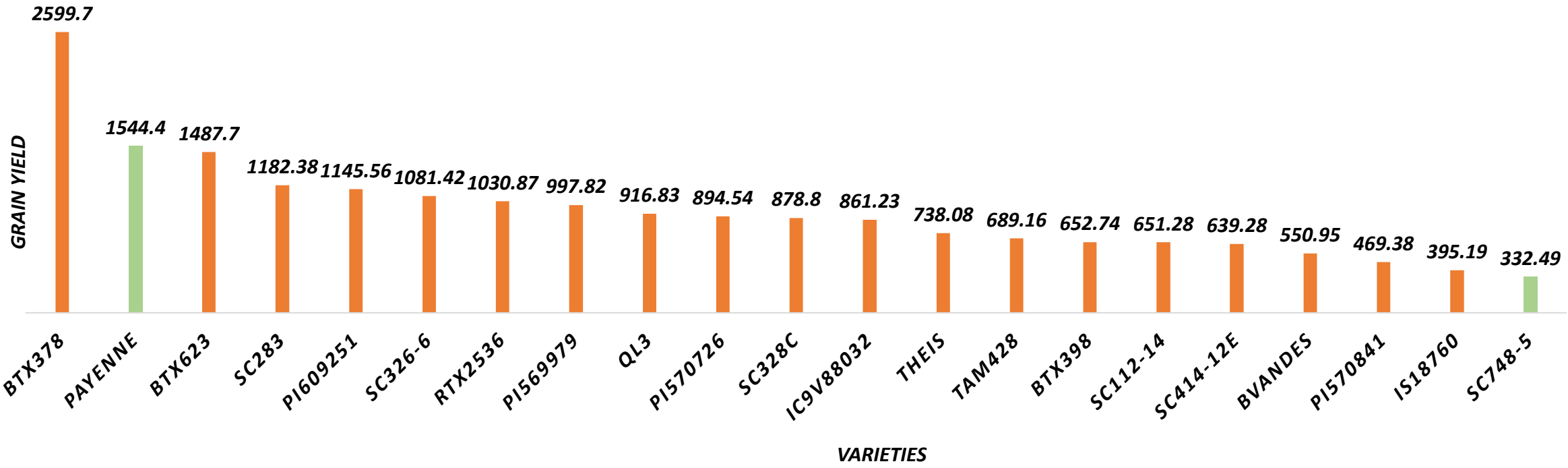
08 varieties showed good tolerance to anthracnose (score 1-3):

- *04 varieties resistant to highly resistant to anthracnose at Bambey and Sefa (score 1-2);*
- *04 varieties moderately resistant to anthracnose;*

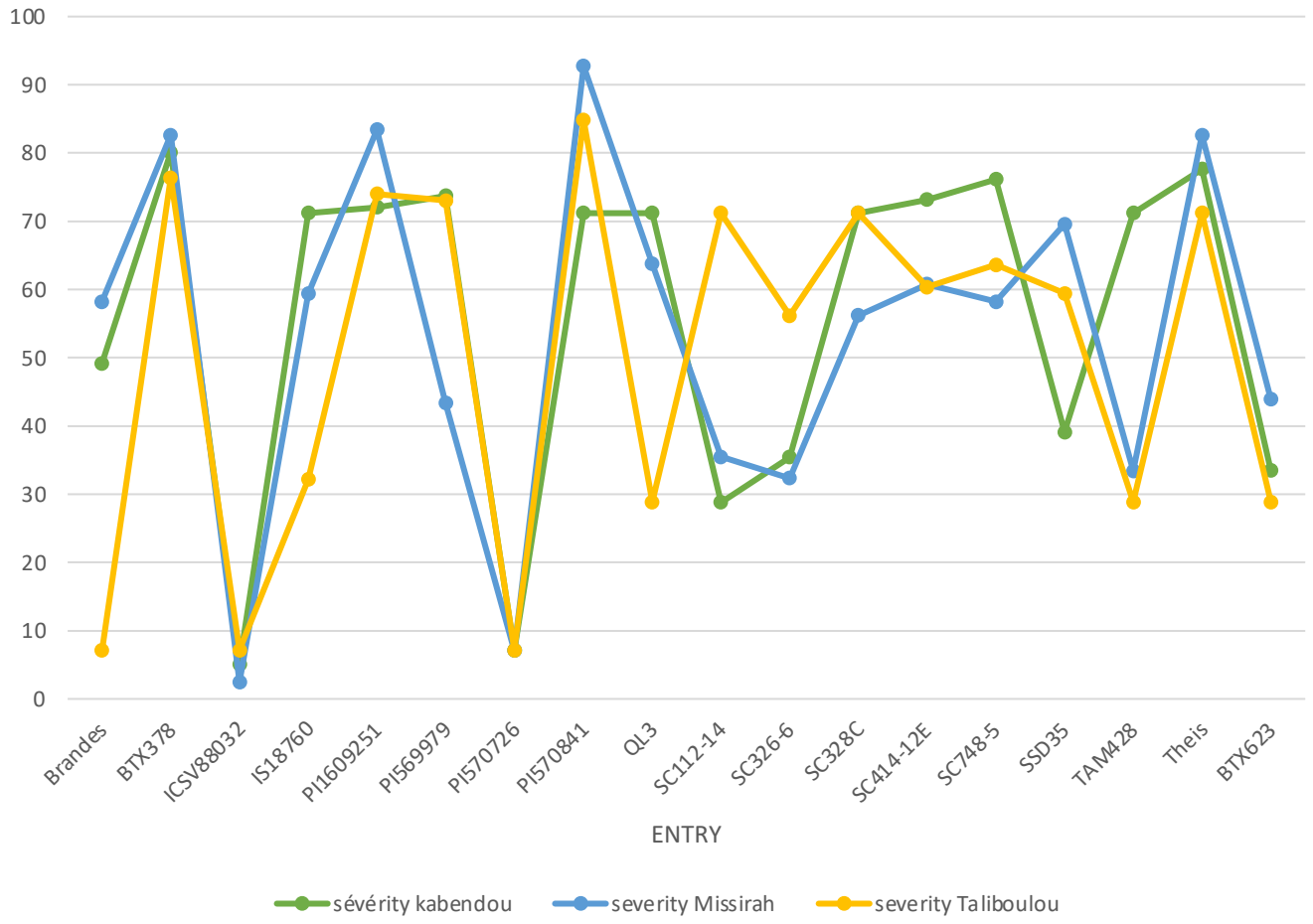
Variety	Anthracnose	
	Bambey-2021	Sefa-2021
PI570726	***1,8***	***1,0***
IC9V88032	***2,6***	***1,0***
SC283	***1,3***	***1,0***
RTx2536	***1,3***	***1,0***
BTx398	***1,7***	***1,0***
BTx623	***3.3***	***3,0***
TAM428	***3,3***	***3,0***
SC748-5	***3,4***	***3,0***

Evaluation of Anthracnose Differentials at ISRA Stations (Bambey, Roff and Sefa)

- 06 varieties with yield ≥ 1t/ha;
- BTx623 and PI609251 high yield and resistance to grain mold;
- RTx2536 and BTx623 high yield and resistance to Anthracnose;



Evaluation of Anthracnose Differentials in hot spots



- 02 out of the 18 differentials were resistant to anthracnose PI570726 and ICSV88032
- SC748-5, the resistance check had more than 55 % severity in the 3 sites;
- BTX-623, the susceptible check had less than 45 % severity in the 3 sites.

Collection of grain mold samples

- 75 samples of grain mold were collected during rainy season 2021 at Séfa and Roff.

- Séfa is in a very humid region(average rainfall 1000 mm) and roff not so humid (average rainfall around 400 mm).

- These samples were dried at room temperature for one week after collection before we start solation of pathogens for diversity.

Next steps

- Test all resistant lines of the populations and the high yielding lines national collection in greenhouse and hot spots for their resistance to main sorghum diseases;
- Workshop with farmers for feed-back;
- Second survey for main sorghum diseases evaluation;
- Screening in hot spots and greenhouse for other diseases (leaf blight)
- Make crosses
- Estimates losses due to diseases and their impact on farmers (with a socio-economist).

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Activities conducted in 2022 in Niger

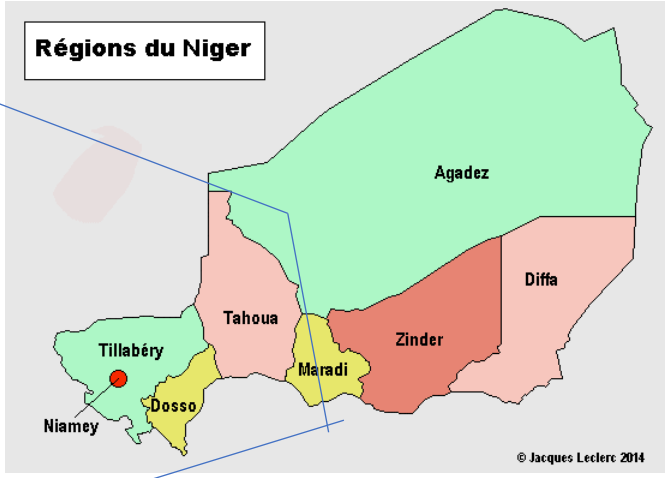
- 2021 annual meeting
- Survey on sorghum foliar and head diseases
- Research station trials
- On-farm test

2021 annual meeting

- Participants: 19 (researchers, extensionists, producers)
- City : Dosso
- Presentation of the project objectives and results for 2020
- Planning of the 2021 activities

Survey on sorghum foliar and head diseases

Zones of study



Méthodology

- In each field, 60 plants at head maturity stage were assessed using a W-shaped pattern.

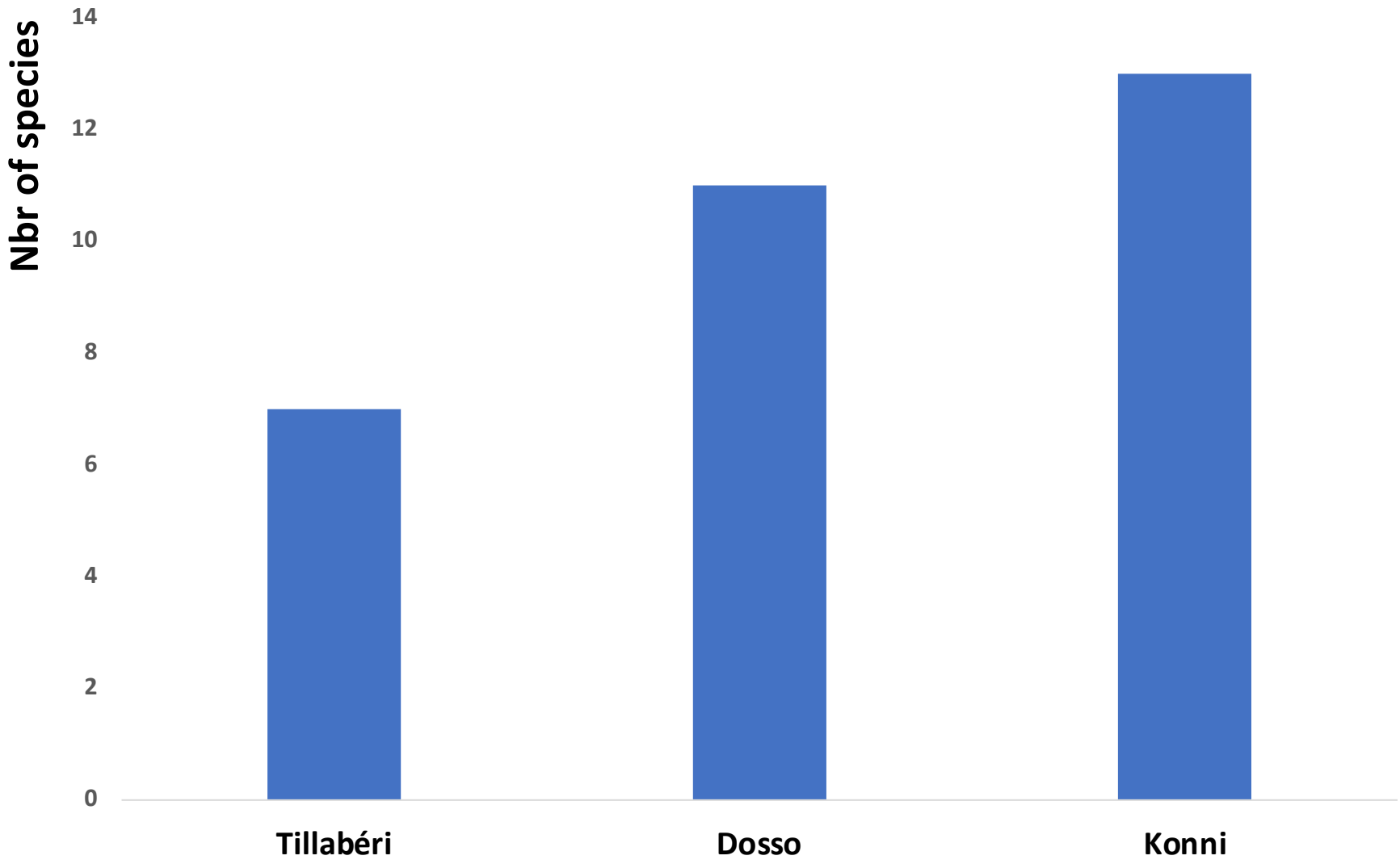
$$\text{Prevalence rate} = \frac{\text{Number of fields with the disease}}{\text{Total number of fields surveyed}} \times 100$$

$$\text{Incidence} = \frac{\text{Number of plants with the disease in a field}}{\text{Number of plants assessed in a field}} \times 100$$

13 Diseases encountered

- Anthracnose
- Rough leaf spot
- Oval leaf spot
- Blight
- Target
- Fusarium
- Loose smut
- Head smut
- Long smut
- Covered smut
- Bacterial streak
- Zonate
- Grain mold

Number of foliar diseases/region



Prevalence of the diseases

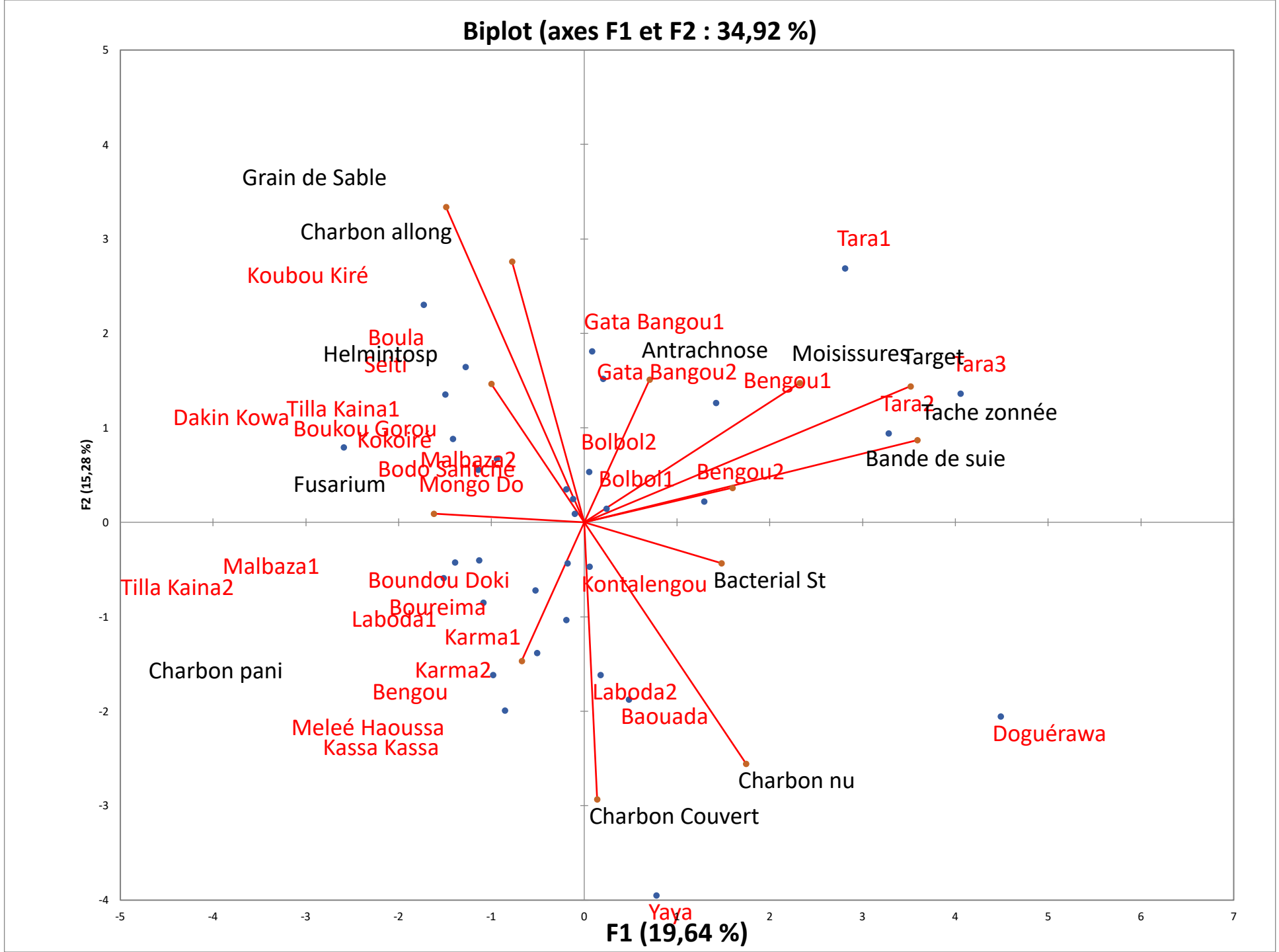
Dosso	Maladies sorgho												
		Bact st	Long smut	Blight	Rough leaf spot	Anthracnose	Oval leaf spot	Covered smut	Loose smut	Target	Grain mold	Fusarium	Zonate	Head smut
Prevalence		25	43.48	56.53	34.78	86.96	69.56	4.36	0	26.09	8.70	4.36	39.13	13.04

Tahoua	Maladies sorgho												
		Bact st	Long smut	Blight	Rough leaf spot	Anthracnose	Oval leaf spot	Covered smut	Loose smut	Target	Grain mold	Fusarium	Zonate	Head smut
Prevalence		8.7	33.33	100	66.67	33.33	83.33	0	0	0	16.67	0	50	33.33

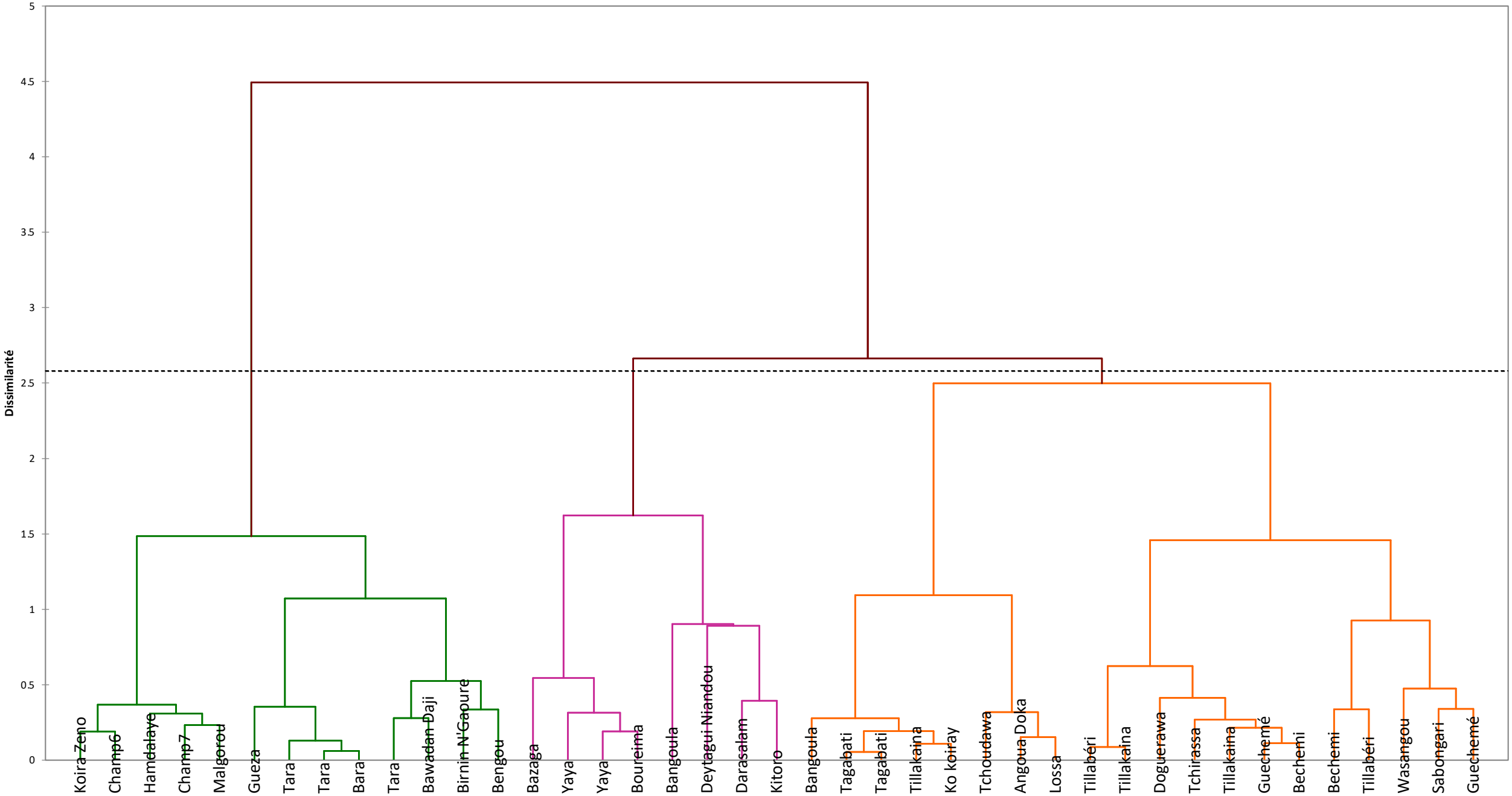
Tillabéri	Maladies sorgho												
		Bact st	Long smut	Blight	Rough leaf spot	Anthracnose	Oval leaf spot	Covered smut	Loose smut	Target	Grain mold	Fusarium	Zonate	Head smut
Prévalence		3	58.33	91.67	50	50	33.33	8	8.33	8	8	3	0	0

Incidence of the sorghum foliar and head diseases in western Niger

	Tillabéri	Dosso	Konni
Antrachnose	100	100	100
Rough leaf spot	80	87	25
Oval leaf spot	100	87	87,5
Blight	70	40	50
Target leaf spot	0	40	12,5
Fusarium	10	6,7	12,5
Loose smut	0	0	25
Head smut	0	27	87,5
Long smut	60	87	75
Covered smut	0	0	25
Bacterial St	0	6,7	12,5
Zonate leaf spot	0	53	50
Grain mold	100	100	87,5


Biplot (axes F1 et F2 : 34,92 %)
F2 (15,28 %)
F1 (19,64 %)
Grain de Sable
Charbon allong
Koubou Kiré
Boula
Seiti
Helmintosp
Dakin Kowa
Tilla Kaina1
Boukou Gorou
Kokoiré
Malbaza2
Bodo Sanichié
Mongo Do
Fusarium
Gata Bangou1
Antrachnose
Gata Bangou2
Bengou1
Moisissures
Target
Tara3
Tara1
Tara2
Tache zonnée
Bande de suie
Bolbol2
Bolbol1
Bengou2
Malbaza1
Tilla Kaina2
Boundou Doki
Boureima
Laboda1
Karma1
Karma2
Bengou
Meleé Haoussa
Kassa Kassa
Charbon pani
Kontalengou
Bacterial St
Laboda2
Baouada
Charbon nu
Charbon Couvert
Doguérawa
Yava

Grouping of the sites according the diseases'importance



Hot Spots for the Major Diseases

- Anthracnose (everywhere)
- Oval leaf spot (Tara, Tanda, Bengou, Doguéraoua)
- Zonate (Dogaraoua, Tara)
- Blight (Kollo, Bengou, Tillabéri)
- Mold (Kollo, Bengou and Tara)
- Head smut (Bazaga, Laboda)
- Long smut (Kollo, Bazaga)

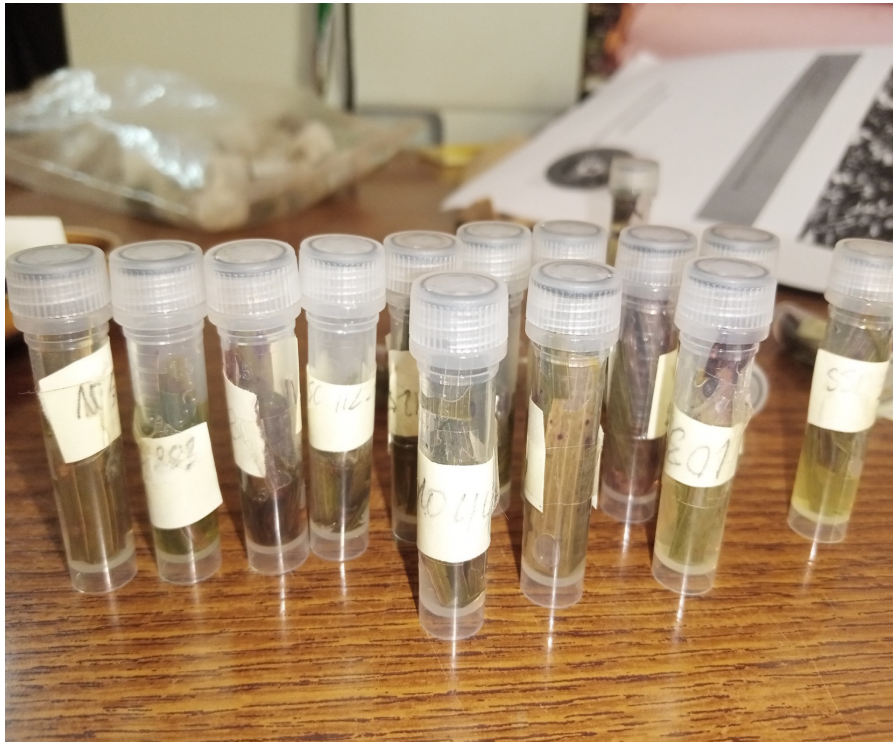
Research station trials at **BENGOU**

Varieties	Antracnose	Blight	Zonate	Grain mold	Oval leaf spot	Target	Rough L Spot	bande de suie	Long smut
Sc 328C	45	15	0	0	1	0	0	0	0
SSD 35	35	12	15	0	0	0	0	0	0
Sépon 82	40	15	0	0	0	0	0	0	22
IRAT 204	15	55	0	0	0	0	0	0	5
RTX 2536	20	10	0	0	0	0	0	0	0
Sc 283	5	0	30	45	0	0	1	0	0
BTX 378	0	5	80	40	0	0	0	0	0
Theis	0	0	5	0	0	2	5	0	0
Sc 326-6	3	0	7	0	0	0	0	0	0
BTX 623	15	0	2	0	60	0	0	0	0
BTX 398	14	5	1	0	0	0	0	0	0
Brandes	15	0	0	0	15	0	0	0	0
PI 570841	15	25	0	0	25	0	0	0	0
IS 18760	38	0	5	0	42	0	0	0	0
PI 569979	0	5	2	0	0	0	0	0	0
SC 112-14	50	0	1	0	20	5	0	0	0
SC 414-12E	40	0	60	0	5	0	45	0	0
SC 748-5	0	35	0	0	0	0	0	0	0
TAM 428	40	5	0	0	10	0	0	0	0
Pt 609251	20	0	35	0	0	0	5	15	0
Soubatoumi	0	25	0	0	0	0	0	0	40

On-farm test at BENGOU

Varietés	Antr	Blight	Zonate	Grain mold	Oval L spot	Rough L spot	Long smut
IRAT 204	20	35	0	15	0	1	20
Mota Maradi	40	0	0	0	40	0	0
sépon 82	0	45	0	0	0	5	0
Far fara karama	45	0	0	0	0	2	5
SC 748-5	0	25	0	25	0	0	0
Soubatomi	0	35	0	0	0	0	5
SSD 35	20	0	35	0	5	0	0

Sending samples of leaves attacked by antrachnose



- Over 60 samples from various regions were collected in October 2021 and sent to College station for DNA extraction

Collection of seeds and panicles for mold isolation

- More than 50 samples were collected from the mature sorghum fields

- These samples are preserved for later use in the isolation of mold fungi

Breeder's lines reaction to sorghum diseases in Kollo

Famille	Anthracnose	Target	Grain mold	LB	Smuts	Zonate	Oval L spot	Rough L spot
P58	**0**	**0**	**3**	**15**	**0**	**0**	**0**	**0**
P33	0	0	30	5	0	1	0	0
P93	0	0	0	10	0	0	0	0
BCS1:119-1	0	0	0	15	0	0	0	0
P91	0	0	30	10	0	0	0	0
P96	10	1	0	10	0	0	0	0
P82	0	0	50	15	0	0	0	0
P39	0	0	60	15	0	0	0	0
P85	0	0	40	15	0	0	0	0
P84	0	0	100	20	0	0	0	0
P86	0	0	60	20	0	0	0	0
P89	0	0	100	20	0	0	0	0
P78	0	0	100	20	0	0	0	0
P81	0	0	100	30	0	0	0	0
P79	0	0	60	10	1	0	0	0
P76	0	0	40	45	0	0	0	0
P77	0	0	50-60	20	0	0	0	0
P71	0	0	100	25	0	0	0	0
P74	0	0	100	20	0	0	0	0
P75	0	0	50-60	25	0	0	0	0
P45	0	0	100	10	0	0	0	0
BCS1:102-1	2	0	100	20	0	0	1	0
P32	0	0	100	10	0	0	0	0

7	asco	LB	Antrac	Moisissu	tacheoval	Zonate	strillebac	Charbon A	Target
sepon	50	100	0	0	0	0	0	0	0
L28 pmr	0	10	0	0	0	0	0	0	0
wassa	0	10	0	0	0	0	0	0	0
L28	25	10	1	0	0	0	0	0	0
irat	20	10	0	0	0	0	0	0	0
1006	20	45	10	0	10	0	0	0	0
1007	0	0	0	60	0	0	0	0	0
1008	2	0	0	0	0	0	2	0	0
1009	0	0	0	50	0	0	0	0	0
1010	45	10	0	0	0	0	0	0	0
1011	10	0	10	0	0	0	0	0	0
1013	20	10	27	0	0	0	0	0	0
1014	0	50	0	0	0	0	0	0	0
1015	0	30	10	0	0	0	0	0	30
1016	0	0	7	0	0	0	0	0	0
1017	0	0	0	43	0	0	0	0	0
1018	0	0	0	40	5	5	0	0	0
1019	10	45	0	0	0	10	0	0	0
1020	0	0	0	0	0	10	10	0	0
1021	15	40	0	0	0	0	0	0	0
1022	20	60	0	30	0	0	0	0	0

BENGOU

1024	0	30	0	0	25	0	0	0
1025	10	30	0	0	0	0	0	0
1026P78	0	0	10	0	0	0	2	0
1027	0	20	0	0	10	0	0	0
1028	0	15	0	0	0	0	0	0
1029	0	10	0	0	0	1	0	0
1030	0	15	0	0	0	0	0	0
1031	0	10	0	0	0	10	0	0
1032	0	20	0	0	0	1	0	0
1033	10	2	0	0	0	1	0	0
1034	15	10	0	0	0	0	0	0
1035	0	10	0	20	0	1	0	0
1036	0	5	0	0	0	0	0	0
1037	20	15	0	0	0	1	0	0
1039	0	5	5	0	2	2	0	0
1040	20	10	0	0	0	0	0	0
1041	10	20	0	30	10	0	0	0
1042	10	20	0	0	5	0	0	0
1043	10	45	10		10	0	0	0
1044	0	20	10		2	0	0	0
1045	0	10	0	0	0	0	0	0
1046	10	0	0	0	10	0	0	0

Major constraints

- End-of-cycle drought in all sorghum production areas

Perspectives

- Isolation of fungi from sorghum leaves and seeds (anthracnose and mold)
- Holding of the programming meeting for the 2022 year activities

FEED THE FUTURE

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

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