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

INNOVATION LAB FOR COLLABORATIVE RESEARCH ON SORGHUM AND MILLET

Innovating science to build the crops of the future

FIVE-YEAR FINAL REPORT

JULY 23, 2013 - JULY 22, 2018

USAID FROM THE AMERICAN PEOPLE

FEED THE FUTURE INNOVATION LAB
Collaborative Research on Sorghum and Millet

KANSAS STATE UNIVERSITY

Feed the Future Innovation Lab for Collaborative Research on Sorghum and Millet

Five-Year Final Report

July 23, 2013 – July 22, 2018

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Front cover photo:
A researcher analyzes a millet trial in Mali.
Photo credit: Nat Bascom

Back cover photos (left to right; bottom):
Women carry millet to thresh in a village in Niger.
Photo credit: Timothy J. Dalton

A woman prepares couscous at the INRAN Food Processing Lab in Niamey, Niger.
Photo credit: Kira Everhart-Valentin

Desalegn Serba, pearl millet breeder at Kansas State University, makes observations of a pearl millet field trial at IER in Mali.
Photo credit: Nat Bascom

Researcher Jack Akata checks on sorghum trials at a CERAAS research station in Bambey, Senegal.
Photo credit: Kira Everhart-Valentin

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EXECUTIVE SUMMARY

This report presents progress over the first five-year phase of the Feed the Future Innovation Lab for Collaborative Research on Sorghum and Millet. The five-year phase of the Sorghum and Millet Innovation Lab (SMIL) began on July 23, 2013 and concluded on July 22, 2018 but follows a thirty-four year history of the INTSORMIL program under the Collaborative Research Support Program (CRSP) mechanism. Kansas State University hosts the Sorghum and Millet Innovation Lab in the College of Agriculture. The program administers a "Leader with Associates" Cooperative agreement with substantive involvement of USAID. The primary goal of the management entity is to develop a portfolio of research activities, combined with human and institutional capacity development, to improve the functioning of the sorghum and millet value chains in target countries. An external advisory board serves a managerial role in the selection, overview and evaluation of projects and advice on programmatic management.

The Sorghum and Millet Innovation Lab funded twelve research projects that were selected through a competitive process: four in Ethiopia and eight targeting West Africa. Seven projects focus on genetic enhancement of sorghum or pearl millet, two on production systems management and two on added-value product development and markets. One project cuts across genetic enhancement and production systems management. Several technological innovations were developed that are being scaled while the breeding programs have generated intermediate innovations. In addition, the management entity was awarded an associate award in Haiti that will terminate in 2019 that is not included in this report.

The Sorghum and Millet Innovation Lab funded, either fully or partially, 68 long-term students. Four of these students received prestigious awards for their accomplishments including the BIFAD award for student excellence. Short-term trainings reached more participants than originally anticipated. These trainees included producers, civil society members (predominantly researchers and students), government representatives and individuals from private sector firms. Producers made up the largest portion of those trained through the Sorghum and Millet Innovation Lab activities. Across the five-year period of the Lab's first phase, approximately 9,750 individuals were trained.

In pursuit of our programmatic objective to build a coalition of science and industry around sorghum and millet where structure and opportunity creates entrepreneurial advances to reduce poverty and hunger, SMIL co-hosted, in collaboration with the University of Pretoria, the first global sorghum conference of this century and the first in over twenty-five years. Held from April 9-12, 2018 in Cape Town, South Africa, the conference drew over 400 participants from 40 countries comprised of researchers, industry professionals, government representatives and development specialists around a broad variety of sorghum-related topics, including food security, value-added products, genetics, global trade, climate-smart agriculture. In addition, a parallel but smaller convening was held on pearl millet. Nearly 90 researchers and stakeholders from across the West Africa pearl millet value chain came together at a regional convening co-hosted by the Sorghum and Millet Innovation Lab on September 4-6, 2018 in Thies, Senegal. Targeted at major actors in pearl millet from Senegal, Niger, Mali and Burkina Faso, the *2018 West Africa Regional Pearl Millet Convening* was also co-hosted by the Centre d'Etude Régional pour l'Amélioration de l'Adaptation à la Sécheresse (CERAAS), and the USDA's NCBA CLUSA's Millet Business Services Project.

The second five-year phase of the program will focus on refining the research project portfolio to target downstream impact through a competitive call for proposal combined with a results-oriented evaluation of activities and projects funded in the first phase.

PROGRAM PARTNERS

UNITED STATES

Cornell University
Integrated Pest Management Innovation Lab-Virginia Tech University
Kansas State University
Kansas State University – Western Kansas Agricultural Research Center, Hays
Purdue University
Texas A&M AgriLife Research
Texas A&M University
USDA-Agricultural Research Service
Virginia Tech University
West Texas A&M University

ETHIOPIA

Ethiopian Institute of Agricultural Research
Asosa Research Center
Bako Research Center
Melkassa Research Center
Pawe Research Center
Sirinka Research Center
Haramaya University
Hawassa University
Holleta Biotechnology Center
Oromia Regional Program
Tigray Agricultural Research Institute
Tigray Regional Program

SENEGAL

Centre d'Etudes Régional pour l'Amélioration de l'Adaptation à la Sécheresse
Centre National de Recherche Agronomique
FAPAL (farmer organization)
Institut Sénégalais de Recherches Agricoles
Institut de Technologie Alimentaire
University Cheikh Anta Diop de Dakar

NIGER

Fuma Gaskiya (farmer organization)
HALAL (farmer organization)
Institut National de la Recherche Agronomique du Niger
International Crops Research Institute for the Semi-Arid Tropics
LSDS (farmer organization)
University of Maradi

MALI
Institut d'Economie Rurale

BURKINA FASO
Institut de l'Environnement et de Recherches Agricoles

HAITI
CHIBAS
Quisqueya University

GERMANY
University of Hohenheim

FRANCE
Centre de Coopération Internationale en Recherche Agronomique pour le Développement

REPUBLIC OF SOUTH AFRICA
University of Pretoria

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PROGRAM GOALS AND OBJECTIVES

The vision of success of the Kansas State University management entity of the Feed the Future Innovation Lab for Collaborative Research on Sorghum and Millet (SMIL) is to become the leading knowledge management center for sorghum and millet science, added-value product development, climate adaptation strategies in dryland areas and impact accounting. This vision builds from three interrelated goals: 1) Our first goal is to build a coalition of science and industry around sorghum and millet where structure and opportunity create entrepreneurial advances to reduce poverty and hunger. We will achieve this goal by building a knowledge network where USAID, Kansas State, land grant universities (LGUs), international collaborators, National Agricultural Research Institutes (NARIs), national universities, and the private sector can contribute their specialized expertise to build resilient and climate-smart sorghum and millet value chains. 2) The second goal of the program is to incubate and nurture a new wave of feed and food products to stimulate demand for sorghum and millet thereby extending economic benefits beyond the farmgate into the broader population. Potential beneficiaries include producers, storage, food and feed processing sectors, and especially consumers. 3) The third goal of the program is to create an economically rationalized business and research investment plan to leverage USAID core financing and attract associate awards and broader donor support.

Activities and Outputs

Activity: Administration

This report presents progress over the first five-year phase of the Feed the Future Innovation Lab for Collaborative Research on Sorghum and Millet (SMIL). The five-year phase of the Sorghum and Millet Innovation Lab began on July 23, 2013 and concluded on July 22, 2018 but follows a thirty-four year history of the INTSORMIL program under the Collaborative Research Support Program (CRSP) mechanism. Despite the successful history of the INTSORMIL program, there were several reorientations when the Sorghum and Millet Innovation Lab began.

Kansas State University hosts the Sorghum and Millet Innovation Lab in the College of Agriculture. The program administers a "Leader with Associates" Cooperative agreement with substantive involvement of USAID. The primary goal of the management entity is to develop a portfolio of research activities, combined with human and institutional capacity development, to improve the functioning of the sorghum and millet value chains in target countries. An external advisory board serves a managerial role in the selection, overview and evaluation of projects and advice on programmatic management. Table 1 presents the management team and external advisory board members. The USAID Agreement Officer's Representative is Dr. Angela Records (initially Dr. Jennifer "Vern" Long).

Table 1. Management entity staff and External Advisory Board Members

Management Entity		**External Advisory Board**
Director	Dr. Timothy J. Dalton	Dr. Barbara Stoecker
Assistant Director	Nat Bascom	Dr. Bettina Haussmann
Program Coordinator	Kira Everhart-Valentin	Dr. Brhane Gebrekidan
Business Financial Specialist	Kim Suther[1]	Dr. Peter Matlon
		Tim Lust

[1]Initially held by Kristen Sanborn

The most dramatic shift in the SMIL program was the focus on three target countries - Senegal, Niger and Ethiopia - as opposed to programmatic activities in twenty-one countries in Africa and Central America under the INTSORMIL program. By focusing activities in three countries, the need for regional coordinating projects was eliminated. In addition, the focus on three countries and their national agricultural research systems created an opportunity to coordinate cross-disciplinary activities within a country and build institutional capacities.

The second shift was to regroup research activities along three areas of inquiry: 1) Genetic Enhancement, 2) Production Systems Management, and 3) Added-value products and marketing. These areas of inquiry are closely related to the previous activities under INTSORMIL. In order to develop the research project portfolio for SMIL, a two-stage competitive call for proposals was issued. The management entity received nearly sixty concept notes covering all areas of inquiry and for both sorghum and pearl millet. The external advisory board, the management entity and USAID evaluated each concept note and selected fifteen to be developed into full project proposals. From these fifteen proposals, ten projects were selected for funding: four in Ethiopia and six in West Africa. Five of the ten projects focused on genetic enhancement, two specifically on production systems management and two on added-value product development. One project cut across genetic enhancement and production systems management. One weakness of the program was the lack of research projects in pearl millet genetic enhancement and this was addressed by soliciting a research project concept note from pearl millet breeders in Senegal, Mali, Burkina Faso and Niger. This concept note evolved into a funded research project led by the senior pearl millet breeder in the target countries. In addition to these research projects, Kansas State University provided funding to support graduate students and research in agricultural economics and in pearl millet improvement, though the recruitment of a breeder based at the Western Kansas Agricultural Research Center, to restart the successful genetic enhancement program established there in the 1970's. Activities and accomplishments for each research project are described in the second section of this report.

Activity: Long-term Training

One of the critical areas of priority for the Sorghum and Millet Innovation Lab throughout the first five years of implementation has been in trainings – both long- and short-term. Under long-term training, which includes a variety of degree-earning program types, the Lab supported (some fully and some partially) 68 trainees between 2014-2018. Among those, 46 were male and 22 were female. Two of the trainings were of agricultural engineers, five were Bachelor's degrees, 30 were Master's degrees, 30 were Ph.Ds. and one was a post-doc.

Student awards

Numerous students trained under Sorghum and Millet Innovation Lab support received awards of recognition for their accomplishments during the Lab's first five-year phase. Some of these awards include:

- *2018 BIFAD Award for Scientific Excellence in a Feed the Future Innovation Lab*
  Laouali Amadou - Université de Maradi - Niger

- *Student Poster Presentation – Sorghum in the 21st Century (2018 Global Sorghum Conference)*
  Fanna Maina - Kansas State University, USA and Institut National de la Recherche Agronomique du Niger (INRAN), Niger

- *People's Choice Award for the Three-Minute Thesis (3MT) - Sorghum in the 21st Century (2018 Global Sorghum Conference)*
  Abdourahmane Diop - Institut de Technologies Alimentaires (ITA), Senegal

- *Best Poster – 2015 Conference on Tropical and Subtropical Agricultural and Natural Resource Management (TROPENTAG)*
  Charles Ikenna Nwankwo - University of Hohenheim, Germany

Activity: Short-term Trainings

Short-term trainings reached more participants than originally anticipated. These trainees included producers, civil society members (predominantly researchers and students), government representatives and individuals from private sector firms. Producers made up the largest portion of those trained by Sorghum and Millet Innovation Lab activities. Across the five-year period of the Lab's first phase, approximately 9,750 individuals were trained, with an increasing gender equity over time.

2014	Projects contracted and launched; no short-term trainings recorded		
2015	Total trainings:	47	
	Individuals trained:	3,013	(38% female, 62% male)
2016	Total trainings:	25	
	Individuals trained:	2,065	(33% female, 67% male)
2017	Total trainings:	29	
	Individuals trained:	2,590	(50% female, 48% male, 2% unknown)
2018	Total trainings:	19	
	Individuals trained:	2,081	(66% female, 34% male)

Activity: Institutional development

Cooperation with our National Agricultural Research Institutes (NARIs) partners in Senegal, Niger and Ethiopia to strengthen their environmental compliance capacity was organized. The SMIL management entity coordinated with the existing environmental compliance resource persons/framework, linked key NARIs staff to additional USAID-led regional environmental compliance training opportunities, organized environmental compliance cross learning for NARIs, and introduced a web based environmental compliance reporting module.

In Ethiopia, initial discussions with NARI partner, the Ethiopian Institute of Agricultural Research (EIAR), centered on the recruitment of an external candidate from the national pool to fill the Sorghum and Millet Innovation Lab country coordinator position. It was envisioned that this person would take on a strategic vision role to interface with the much wider sector of development actors and the private industry, identify new partnership and financing opportunities within Ethiopia and the region to enhance impact while working closely with EIAR structures. The EIAR later requested that this position be revised, the external search was stopped and the position was filled internally.

Activity: Collaboration and Networking

During this five-year phase of the program, the SMIL program has facilitated collaboration between six U.S. universities, six national agricultural research organizations (including USDA), four regional research programs, one CGIAR center, seven international universities, four private sector firms, five farmer organizations and the French agency for international agricultural research and development (CIRAD). The program led a global conference on sorghum research and development.

GLOBAL SORGHUM CONFERENCE

In a capstone component of the program's first five years, the Sorghum and Millet Innovation Lab spearheaded the organization and execution of *Sorghum in the 21st Century: Food, Feed and Fuel in a Rapidly Changing World*, a global sorghum conference that took place on April 9-12, 2018 at the Century City Convention Center in Cape Town, South Africa. As the first global conference on sorghum in over 25 years, the event saw the attendance of more than 400 international researchers, industry professionals, government representatives and development specialists around a broad variety of sorghum-related topics, including food security, value-added products, genetics, global trade, climate-smart agriculture and more (Figure 1). The conference delegates represented 40 different countries from around the world.

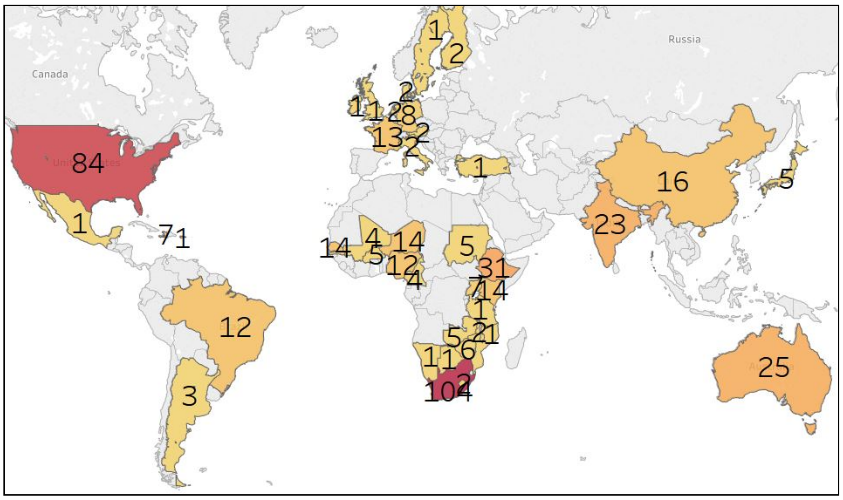

Figure 1. Global sorghum conference participants by country of origin

The conference was co-hosted by the University of Pretoria and offered a unique opportunity to convene around crucial issues for the global sorghum community. It was sponsored by numerous international and local organizations and institutions including the United States Agency for International Development (USAID), the United States Department of Agriculture (USDA), the Australian Sorghum Conference Committee, the United Sorghum Checkoff Program, the McKnight Foundation and the South African Department of Science and Technology and National Research Foundation, among others. The aim was to bring the global community together to establish priorities and collaboration that will lead to key developments in sorghum in the years to come. More information about the Sorghum in the 21st Century conference can be found at: https://21centurysorghum.com/.

2018 WEST AFRICA REGIONAL PEARL MILLET CONVENING

Nearly 90 researchers and stakeholders from across the West Africa pearl millet value chain came together at a regional convening co-hosted by the Sorghum and Millet Innovation Lab on September 4-6, 2018 in Thies, Senegal. Targeted at major actors in pearl millet from Senegal, Niger, Mali and Burkina Faso, the *2018 West Africa Regional Pearl Millet Convening* was also co-hosted by the Centre d'Etude Régional pour l'Amélioration de l'Adaptation à la Sécheresse (CERAAS), and the USDA's NCBA CLUSA's Millet Business Services Project.

The objective of the convening was to provide a forum for the exchange of needs and opportunities by pearl millet stakeholders (including input suppliers, farmers, processors, etc.) with agricultural researchers focused on technological innovation. Over three days, the participants presented current pearl millet value chain activities in several West African nations and discussed technological bottlenecks to improved productivity and profitability of their activities. The groups broke out across subareas and worked together to prioritize the most pressing issues in the West African pearl millet industry. The findings from those breakout groups were made available publicly and will be used to drive future research in the area. While the convening took place in the first month of the second phase of the program, nearly all planning fell within the first phase.

Additional information can be found at: http://www.k-state.edu/smil/whatwedo/pearl_millet/index.html.

Activity: Private Sector Engagement

The SMIL management entity engaged the private sector at an early stage of the technology development in the following ways:

- Direct research cooperation with multiple farmer cooperatives and small scale seed companies in West Africa.

- Engaging food manufacturing companies in Addis Ababa and Hawassa (Hilina Foods/GUTS) through meetings and production site visits together with research teams to foster pathways and partnerships for eventual uptake of food technologies.

- Capacity strengthening of small- to medium-sized companies to produce value-added products and strengthen markets. A Kansas Department of Agriculture (KDA) trade mission to Ethiopia was facilitated to engage EthioGreen a gluten-free teff and sorghum food processing company selling to the domestic and international market. The KDA then provided support for a reverse trade mission in January 2017 for Rahel Mogos, owner of EthioGreen, to visit multiple institutions in Kansas including the Sorghum and Millet Innovation Lab, KDA, American Baking Institute (AIB), a Kansas State Legislator and NuLife Markets.

- Engagement of large scale processors in Senegal in the utilization and uptake of an efficient "economical couscous" production technology targeting both domestic and export markets.

Activity: Gender integration efforts

GENDER STUDY: SORGHUM PRODUCTION AND UTILIZATION IN ETHIOPIA

During its first five-year phase, the Sorghum and Millet Innovation Lab commissioned a gender study led by gender consultant Yeshi Chiche in Ethiopia. The purpose of the study was to assess gender roles and sorghum production/utilization by region in Ethiopia. In 2017, Ms. Chiche and the regional research teams implemented village-level data collection in six different regions using focus group interviews and rapid rural appraisals. The data from those interviews was aggregated into individual regional reports and presented at a project debrief meeting in August 2017. The data was later presented at the *Sorghum in the 21st Century* global sorghum conference in April 2018 and the reports were professionally edited in the fall of 2018 for further distribution and publication.

RESEARCH PROJECT GENDER INTEGRATION

In 2017, all projects were required to build a gender integration plan into their research project operational plans in an effort to have a more effective approach to gender integration across the program. These plans included practical ways in which considerations for gender could be incorporated into existing project activities. Since that time, projects continued to make progress in their approaches to female scientist recruitment, farmer trial and consumer feedback session structures and overall need assessment priorities.

Activity: Utilization of Outputs - Scaling Technology Packages

The multi-faceted SMIL research program portfolio is delivering technology packages to the marketplace. At present, the SMIL program has numerous technologies in process at a phase I (*Under research*), II (*Under field-testing*), III (*Available for scaling*), IV (*Actively being scaled*) level with other technologies that will continue to develop. Phase III technology packages that are now available for scaling include:

- Registered sorghum germplasm with resistance against the sugarcane aphid for utilization by global breeding teams.
- A white sorghum hybrid with strong yield performance for uptake in lowland production areas of Ethiopia.
- Seed ball formulation, production, and planting techniques to reduce risk and improve yield for pearl millet farmers in the Sahelian zone of West Africa.
- An integrated pest management technology with mass rearing of naturally occurring beneficial parasitoid wasps and timely release at a community level to control the millet head miner in West Africa.
- A more efficient lower cost process to produce "economical couscous" for use in large-scale production in Senegal.

The SMIL management entity will continue to play a strategic role to facilitate enabling environments that support the further scaling of technology packages. This will involve broad based partnerships linked to the marketplace and defining pathways to move technology packages to scale. A technology catalog will be developed to feature technology packages available for scaling.

Activity: Communications

While staffing resources were limited throughout the first five-year phase, the Sorghum and Millet Innovation Lab placed high priority on strategically addressing effective communications around program activities and initiatives. This included periodic e-blasts to a Lab distribution list of more than 1,000 contacts, submissions to Feed the Future e-newsletters on Lab initiatives, a visible presence at numerous U.S. and international meetings and conferences, involvement in University-wide educational and promotional opportunities around research and the publication and distribution of comprehensive activities and impacts reports highlighting Lab accomplishments.

Some key highlights of the Lab's external communications can be seen at the following links:

FEED THE FUTURE E-NEWSLETTERS

"Giving Seeds an Added Boost to Survive Harsh Sahelian Climates" - https://www.feedthefuture.gov/article/giving-seeds-an-added-boost-to-survive-harsh-sahelian-climates/

"Kansas State University Battles Sorghum's Newest Enemy with Science" - https://www.feedthefuture.gov/article/kansas-state-university-battles-sorghum-s-newest-enemy-with-science/

"Revolutionizing a Critical Crop for Millions in Africa" - https://www.feedthefuture.gov/article/revolutionizing-a-critical-crop-for-millions-in-africa/

ACTIVITIES AND IMPACTS REPORTS

2014 Activities and Impacts Report - http://www.k-state.edu/smil/docs/2014%20Activities%20and%20Impacts%208-15%20Updated.pdf

2015 Activities and Impacts Report - http://www.k-state.edu/smil/docs/2015%20Activities%20and%20Impacts_Update.pdf

2016-2017 Activities and Impacts Report - http://www.k-state.edu/smil/docs/Program%20Update%202017%20PDF.pdf

FUTURE CHALLENGES AND OPPORTUNITIES

The Sorghum and Millet Innovation Lab was awarded an extension until July 19, 2023. During the second five-year phase, the program will promote research and development activities that will be impactful to end-users whether that be producers, consumers, processors or the next generation of scientists. The following section of the report is divided into project-specific reports on activities and achievements written by each principal investigator. These reports will provide important input into shaping the portfolio of activities funded in the second phase.

The management entity philosophy is one of adaptation to emerging challenges and opportunities combined with a result-based analysis of each project's contribution to national and regional agricultural objectives. The project reports provide one piece of information into the results-based analysis in relation to priorities. Open and competitive calls for research and development activities will be held in targeted areas of need, for example in the pearl millet value chain and plant pathology.

In addition to the evaluation and development of a research project profile, the management entity will continue to facilitate technology transfer in a strategic way. The management entity has been proactive in linking projects with the next users of information and technologies, in developing networks and awareness of programmatic activities and in the marketing of innovations. The program will build upon long-term training achievements by empowering those graduates to be productive as they return to their host institutions.

OVERVIEW OF ACTIVITIES, ACCOMPLISHMENTS, UTILIZATION OF RESEARCH OUTPUTS, FURTHER CHALLENGES AND OPPORTUNITIES

In order to provide the most comprehensive overview of the Sorghum and Millet Innovation Lab's research accomplishments, each principal investigator was requested to provide a final technical report outlining the activities, accomplishments, outputs challenges and opportunities that emerged from their research project throughout the five-year period.

ETHIOPIA

COMBINING HIGH DIGESTIBLE PROTEIN TRAIT WITH WAXY/HETEROWAXY ENDOSPERM TRAITS TO DEVELOP SUPERIOR FUNCTIONALITY IN SORGHUM FOR FOOD APPLICATIONS IN ETHIOPIA

1 – PRINCIPAL INVESTIGATOR

Joseph M. Awika - *Texas A&M University, USA*

2 – RESEARCH TEAM

Co-Investigators:
Kebede Abegaz & Berhanu Abate - *Hawassa University, Ethiopia*
John Taylor - *University of Pretoria, South Africa*
Henriette L. de Kock - *University of Pretoria, South Africa*
William Rooney - *Texas A&M University, USA*

Partners:
Ethiopian Institute of Agricultural Research (EIAR), Melkasa, Ethiopia
Small and Medium Enterprise in Hawassa – bakeries and injera processors
Bless Laboratories, Addis Ababa, Ethiopia
Hilina Foods PLC, Addis Ababa, Ethiopia
BGI Ethiopia – brewing company, Addis Ababa, Ethiopia (has a branch in Hawassa)
Guts Agro Industry, Hawassa, Ethiopia

3 - PROJECT GOALS & OBJECTIVES

The long-term goal of this project is to improve sorghum value and utilization as a food ingredient for food and nutrition security in Ethiopia. Two major bottlenecks that limit sorghum utilization for food in Ethiopia are its inadequate functionality as a food ingredient and lower protein nutritional quality relative to other cereal grains. These characteristics are related to sorghum protein body structure. Texas A&M recently developed hard endosperm sorghum lines and hybrids that combine an altered protein body mutation (commonly called highly digestible protein (HPD) sorghum) with waxy and heterowaxy starch traits (WX) targeted for bioethanol production. We hypothesized that the HPD-WX combination would significantly enhance sorghum endosperm functionality in various food products relevant to Ethiopian consumers, due to synergistic interaction of the altered protein structure with waxy starch. Furthermore, because the HPD is commonly associated with higher protein digestibility and lysine content, we expected these improved sorghums to have higher protein nutritional quality. In this 'proof of concept phase', three specific objectives for this project were to:

1. Establish the effect of combining waxy-heterowaxy (WX) with HPD sorghum traits on dough and batter rheology, food processing, and quality profile of selected traditional and commercial grain-based food products popular in Ethiopia,

2. Establish the suitability of the WX-HPD sorghum hybrids for malting and commercial brewing, and

3. Evaluate the performance and adaptation of the WX-HPD sorghum hybrids in Ethiopia.

4 - OVERVIEW OF ACTIVITIES

Objective 1: Improved sorghum functionality in foods

1. *Physico-chemical attributes of improved sorghums relative to normal varieties*. The rationale for this task was to determine how the HPD-WX trait affects grain quality characteristics known to impact grain processing and product quality, as well as how growing environment affects these attributes. This would enable us to predict the functionality of these grains across multiple environments and scientifically explain their behavior in products.

   Key parameters measured included grain physical quality (hardness, density, milling behavior, etc.), endosperm functional characteristics (starch pasting properties, water absorption, viscosity parameters), grain protein content and amino acid profile.

   Above parameters were measured on more than 80 sorghum lines grown in 2 locations in Texas (4 years), and 3 locations in Ethiopia (2 years). This provided robust data to establish the functional potential of the sorghum lines and their stability across environments.

2. *Functionality of improved sorghums in traditional and modern foods*. The task aimed to establish the actual performance of the HPD-WX sorghums in target products most relevant to Ethiopian consumers in both urban and rural settings. We tested this by substituting sorghum for the primary grain in various products, primarily targeting batter and dough-based systems. Four sorghum endosperm types were compared: Normal non-waxy (control), normal waxy, HPD non-waxy, HPD-waxy. These tests were done *both in laboratory, and in collaboration with small scale processors* in Hawassa, Ethiopia.

   Injera: being the predominant staple in Ethiopia made primarily with teff, an increasingly expensive grain, functional properties of the improved sorghums in injera is highly relevant to both consumers and sorghum producers. Because injera processing is highly variable and non-standardized, we spent the first 2.5 years developing standardized and reproducible protocols to obtain predictable data that accurately reflects the performance of the sorghum lines. Once protocols were standardized, we tested sorghum substitutions at 0 – 100%. Sorghums were tested for both processing performance, and product sensory and instrumental quality.

   Pancake; this is a non-fermented equivalent of injera, made primarily from wheat and popular in urban Ethiopia and many parts of the world. We tested sorghum substitutions at 0 – 100%.

   Bread; wheat-based bread is highly popular in Ethiopian urban areas, yet wheat is largely imported and is an expensive ingredient. Substitution of sorghum even at modest levels of 5-10% without impacting product quality would significantly benefit consumers and processors while saving the government foreign exchange. We tested sorghum performance in breads at 10 – 20% substitution using both traditional (sour-dough) and modern (yeast) fermentation methods.

Snacks; various fried/baked snacks in Ethiopia are based on wheat. We used cookies as a model to test the functional properties of the improved sorghums in such products at 0 – 50% substitution.

3. *Nutritional properties of improved sorghums in food products*. The rationale was to determine whether the HPD trait actually translates into improved protein digestibility and lysine profile in target food products. Protein digestibility of both raw and cooked sorghum (86 lines), as well as processed injera were tested using *in vitro* pepsin assay. Lysine content of select lines (14) were also determined using HPLC.

Objective 2: Improved sorghum functionality for malting and brewing

Malting and brewing are among the most promising industrial uses of sorghum in Ethiopia and other African countries where barley import is costly. The primary goal is to expand market opportunities for sorghum farmers. In this project phase, our primary goal was to test malting characteristics of the improved sorghum lines. Four sorghum lines that encompass the different endosperm traits were compared to commercial sorghum control. Key quality parameters measured included germination efficiency, extract (malt solubilization), amylase and proteolytic activities, wort fermentable sugars and free amino nitrogen. Barley malt was used as a positive control.

Objective 3: Agronomic performance of improved lines in Ethiopia and Texas

Because these new sorghum lines are undergoing genetic improvement and have primarily been restricted to testing in Texas, it was extremely important to demonstrate their adaptability to key sorghum producing environments in Ethiopia. Thus, in addition to on-going annual field testing in Texas (4 years), we evaluated agronomic performance of 28 improved sorghums in 3 environments in Ethiopia over 2 years. The agronomic evaluation was done in collaboration with EIAR using their test plots in Derashe/Gidole (Southern Ethiopia), Meiso/Haraghe (Eastern Ethiopia), and Kobo/Raya (Northern Ethiopia). Through this Objective, we were also able to obtain grain for the tasks described under Objective 1.

Capacity building: Graduate training

Four individuals were trained (3 male, 1 female):

1. Two faculty members at Hawassa University underwent doctoral training at University of Pretoria (Mr. Abadi Mezgebe) and Texas A&M University (Mr. Tadesse Teferra).

2. Two students (Ms. Loza Mengistu, and Mr. Getahun Adane) underwent MS training at Hawassa University.

Capacity building: *Short term*

1. April 2015, Dr. Abegaz and Mr. Mezgebe participated in a 3-day training that included other African scientists, conducted in S. Africa and hosted by University of Pretoria. Professor Taylor and Awika conducted the training. Training involved sorghum milling, analysis, and processing.

2. January 2016, 11 Hawassa university staff were trained (by Awika) on grain and dough quality analysis, as well as use of newly acquired dough mixing performance analyzer (Mixogram).

3. March/April 2016, 6 women entrepreneurs involved in injera processing participated in sensory training at Hawassa University. An additional 36 individuals were involved in sensory testing of injera.

4. January 2017, 9 Hawassa university staff were trained (by Awika) on food moisture and water activity analysis techniques in relation to food quality and safety using analytical equipment purchased using project funds.

5. In July 2017, a one-week workshop at Hawassa University trained 12 staff from Hawassa University and representative of the local processing and brewing industry. The training included in-depth hands-on sensory evaluation techniques for various food products (Professor De Kock, Univ of Pretoria), grain processing and quality testing (Awika, Texas A&M), and malting and malt quality evaluation (Mr. Mezgebe, Hawassa/Pretoria). At the end of this workshop, a product showcase that included injera, bread, and cookies made by local food processors, containing optimal substitutions of sorghum was presented to 23 university administrators, government officials, and private sector representatives, and the media. This **event was covered on National Television and press**.

Capacity building: *Equipment*

Three pieces of equipment were purchased for Hawassa University;

1. Mixograph

2. Moisture analyzer

3. Water activity meter

These are among the basic instruments essential in a grain quality laboratory, and are essential in enhancing Hawassa University's capacity to conduct grain quality research and quality testing. The equipment are currently used for grain analysis and undergraduate/graduate student training.

5 - ACCOMPLISHMENTS

A. Achievements by project objectives

Objective 1: Improved sorghum functionality in foods

Physico-chemical attributes of improved sorghums relative to normal varieties

Table 1. Mean values of physical and starch pasting properties of experimental sorghum lines

Sorghum type	N	Hardness index	TKW (g)	True density (g/cc)	Seed Size (mm)	WSI (%)	Pasting properties	
							Peak viscosity (cP)	Final viscosity (cP)
Control HPD	2	29.7[b]	29.7[a]	1.35[bc]	2.43[b]	5.92[d]	2120[cd]	2863[b]
Control LPD	2	82.5[a]	25.1[b]	1.38[a]	2.5[ab]	4.48[f]	1850[d]	3757[a]
HPD, N	34	68.3[a]	25.7[b]	1.34[c]	2.51[b]	**5.28[e]**	1930[d]	**3032[ab]**
LPD, N	8	80.7[a]	29.9[a]	1.39[a]	2.80[a]	**4.68[f]**	1928[d]	**3460[a]**
HPD, hWX	6	77.1[a]	21.9[c]	1.37[abc]	2.41[b]	**6.63[c]**	2440[c]	**3034[ab]**
LPD, hWX	2	82.2[a]	30.7[a]	1.38[a]	2.60[ab]	**5.72[de]**	1433[e]	**2551[bc]**
HPD, WX	10	77.6[a]	25.7[b]	1.37[ab]	2.62[ab]	**8.25[a]**	3027[a]	**2921[b]**
LPD, WX	6	77.1[a]	31.2[a]	1.36[bc]	2.51[b]	**7.70[b]**	2905[b]	**2272[c]**

TKW = thousand kernel weight; WSI = water solubility index; HPD = high digestible protein body mutation present (based on FE-SEM); LPD = normal protein body (wild type); N = non-waxy, WX = waxy, hWX = heterowaxy; values are means ± SEM; values with different letters in the same column are significantly different ($p<0.05$, Tukey HSD). *Paired comparisons of interest (HPD vs LPD) are color-coded for simplicity.*

1. Improved HPD & HPD-WX lines had hardness scores and physical properties comparable to commercial sorghum, and significantly better than control HPD sorghum (Table 1).

2. HPD trait improved water solubility index of sorghum, suggesting improved ability to interact with other ingredients during food processing. Combined HPD/WX traits improved the water solubility index further, indicating enhanced potential functionality.

3. HPD trait affects starch viscosity profile differently; HPD mutation generally reduced final viscosity of normal starch, but increased the final viscosity of waxy starch. This suggests uniquely distinct interaction mechanisms with the different starch structures, with important implications to food processing and quality. *Mechanisms behind the interactions should be investigated.*

Functionality of improved sorghums in traditional and modern foods

Table 2: Ethiopian Sensory Score of Injera from Four Sorghum Endosperm Traits blended with teff

Treatment ratios	Color	Rollability	Sauce Scooping	Sourness	Taste	Non-sticking	Even eyes	Bottom eyes	Overall acceptability
KT100	8.15[a]	8.17[a]	7.90[a]	7.45[ab]	8.07[a]	7.95[a]	8.00[a]	8.07[a]	8.12[a]
WHD50:KT50	7.00[bcd]	7.15[bcde]	7.22[abcde]	7.32[abc]	7.57[ab]	7.35[abc]	7.50[ab]	7.62[ab]	7.97[ab]
WHD80:KT20	6.27[c]	6.52[de]	6.62[cdef]	6.17[de]	6.47[cde]	6.77[cde]	6.60[cd]	6.30[ef]	6.95[de]
WHD100:KT0	5.52[f]	5.75[f]	6.02[f]	5.75[e]	6.22[de]	5.92[f]	5.77[e]	6.00[f]	6.07[f]
WHDh50:KT50	7.57[ab]	7.80[ab]	7.87[a]	7.65[a]	7.80[a]	7.93[a]	8.02[a]	8.17[a]	8.32[a]
WHDh80:KT20	7.17[bc]	7.42[ab]	7.40[abc]	7.05[abc]	7.15[abc]	7.25[abcd]	7.40[abc]	7.67[ab]	7.67[abc]
WHDh100:KT0	6.42[cde]	6.50[def]	6.57[def]	6.57[c]	6.50[cde]	6.70[cde]	6.80[bcd]	6.75[cdef]	7.02[cde]
WLD50:KT50	7.17[bc]	7.30[bc]	7.47[ab]	7.25[abc]	7.12[abc]	7.27[abcd]	7.22[bcd]	7.47[abc]	7.62[abc]
WLD80:KT20	6.77[bcd]	7.05[bcde]	7.02[bcde]	6.67[cd]	7.00[bcd]	6.62[def]	6.92[bcd]	7.05[bcd]	7.25[cde]
WLD100:KT0	6.02[ef]	6.40[ef]	6.45[ef]	6.12[de]	6.07[e]	6.37[ef]	6.80[bcd]	6.40[ef]	6.55[ef]
NLD50:KT50	6.82[bcd]	7.15[bcde]	7.32[abcd]	7.22[abc]	7.22[abc]	7.20[bcd]	7.12[bcd]	7.42[abcd]	7.52[bcd]
NLD80:KT20	6.70[cde]	7.17[bcd]	7.15[abcde]	6.97[abc]	6.85[bcd]	6.95[cde]	7.22[bcd]	7.25[bcd]	7.17[cde]
NLD100:KT0	6.27[def]	6.65[cde]	6.80[bcde]	6.80[bcd]	6.67[cde]	6.57[def]	6.55[de]	6.70[def]	6.92[de]

Same letter within column not significantly different at $p<0.05$. KT= kuncho teff, WHD= waxy high digestible, WHDh= hetro-waxy high digestible, WLD= waxy low digestible, NLD= non-waxy low digestible. *Top performing sorghum:teff blends shaded in green vs control in blue.*

1. Waxy trait improves sorghum performance in injera as partial substitute for teff at 50% (Table 2).
2. Combined waxy-HPD sorghum produced the best injera at 50% teff substitution; indicating HPD trait interacts with WX trait to improve sorghum functionality.
3. 50% HPD-WX sorghum injera was similar to or better than 100% teff injera in Ethiopian sensory evaluation.
4. Injera processors liked the rapid fermenting nature of HPD/WX sorghums (24 h vs 48 h+ for teff).

Table 3: Bread volume and firmness (texture) of pan bread substituted with whole sorghum flour

Sorghum flour	LPD Control sorghum		HPD Normal		HPD Waxy	
	Loaf volume (cm³)	Firmness (N)	Loaf volume (cm³)	Firmness (N)	Loaf volume (cm³)	Firmness (N)
Control 0%	742 ± 38	175 ± 33	742 ± 38	175 ± 33	742 ± 38	175 ± 33
Test 10%	633 ± 38	272 ± 46	692 ± 14	176 ± 18	683 ± 25	293 ± 8.7
Test 20%	600 ± 25	451 ± 43	616 ± 38	349 ± 5.4	608 ± 14	371 ± 8.1

Values ± sd. Refined bread wheat flour was substituted with whole grain sorghum flour at levels indicated. LD = commercial food grade sorghum; IHD = improved high digestible protein sorghum.

1. HPD trait improved sorghum performance as a partial substitute for wheat in bread; better volume and texture than LPD sorghum (Table 3).

2. Waxy trait did not improve HPD sorghum performance in dough/bread.

Nutritional properties of improved sorghums in food products

Table 4. Comparison of in vitro protein digestibility and qualitative field emission scanning electron microscopy (FE-SEM) method for identifying presence of 'high digestible' (HPD) protein body mutation in hard endosperm sorghum

Sorghum Lines	Means Protein Digestibility (%)		Imaging Identification	Lysine, (%) Total Protein
	Raw	Cooked		
Control HPD	80.1[a]	66.0[a]	+	2.54[A]
Experimental HPD range	61.3 – 77.0	35.0 – 67.0	+	1.89-2.57
Mean, HPD (n=46)	69.4[b]	57.6[b]	+	2.31[B]
Experimental LPD	46.2 – 74.4	36.5 – 61.7	-	1.71-2.14
Mean, LPD (n=26)	61.7[c]	45.6[c]	-	1.96[C]

HPD = 'high digestible' mutation qualitatively identified using FE-SEM (+); LPD = wild type protein body structure qualitatively identified using FE-SEM (-); letters in column denote significant difference ($p<0.05$, Tukey HSD).

1. *In vitro* pepsin assay cannot reliably detect HPD mutation in hard endosperm sorghum.

2. New, simpler, qualitative field emission scanning electron microscopy method for detecting HPD sorghum developed and validated.

3. HPD sorghums have on average higher raw and cooked protein digestibility than wild type.

4. Cooked protein digestibility decreased more in LPD (26%) vs improved HPD sorghum (17%).

5. Improved HPD sorghums had on average significantly higher lysine content than LPD sorghums.

Objective 2: Improved sorghum functionality for malting and brewing

Table 5. Quality attributes of malt prepared from waxy and high protein digestibility sorghum lines germinated at medium moisture level and of commercial barley and sorghum malt references

Sorghum line	Malt Quality				Malting Quality		
	α –amylase (CU/g db)	β - amylase (BU/g db)	HWE [°P] (%, db)	FAN (mg/100 g db)	Malting loss (%)	Starch loss (%)	Protein loss (%)
NWND	113.2[e]	4.0[de]	51.1[bc]	76.3[bc]	11.0[a]	8.4[a]	2.61[a]
NWHD	102.3[e]	4.0[ef]	50.5[bc]	99.6[e]	11.2[a]	10.5[abc]	4.98[c]
WHD2	168.5[h]	4.2[de]	64.2[ef]	80.6[cd]	13.1[a]	14.1[d]	4.85[c]
WND3	136.3[g]	4.8[e]	62.0[de]	85.0[d]	14.3[a]	14.7[d]	7.21[d]
SMC	26.0[a]	0.6[a]	36.2[a]	26.2[a]	Not applicable		
BMC	131.2[fg]	12.0[f]	70.4[f]	83.5[d]			

Columns with different letters significantly different ($p < 0.05$). CU (Ceralpha Unit), BU (Betamyl-3® Unit; db (dry basis); NWND (Non-waxy-normal protein digestibility); NWHD (Non-waxy - high protein digestibility), WHD (waxy-high protein digestibility), WND (waxy-normal protein digestibility), SMC = commercial sorghum malt), BMC = commercial barley malt.

1. All experimental sorghums performed dramatically better than commercial sorghum malt, likely due to absence of tannins in the experimental lines (Table 5).

2. Waxy (WX) trait significantly improved sorghum malt performance, HPD effect was not significant.

Objective 3: Agronomic performance of improved lines in Ethiopia and Texas

Table 6. Agronomic performance of 13 entries from 24 TAMU IHD entries in Ethiopia under drought stress

Entry	Type	DM	PH (cm)	Heads harvested	Grain yield kg/ha	Yield rank	Overall score
1	Hetero waxy	96	92	16	1740	12	2.7
2	Normal	96	105	18	2102	1	2.7
4	Normal	98	117	15	1773	9	2.8
5	Normal	99	122	14	1867	8	2.5
6	Hetero waxy	104	113	16	2096	2	2.5
7	Hetero waxy	96	91	17	1886	6	3.3
13	Hetero waxy	93	105	14	1876	7	3.0
14	Hetero waxy	100	125	11	1751	11	2.5
19	Normal	97	113	17	1954	4	2.7
22	Hetero waxy	96	123	14	2000	3	3.5
25	Normal	96	105	14	1766	10	2.8
29 (ESH-3)	Normal	99	133	14	1686	13	2.3
30 (ESH-1)	Normal	101	119	15	1897	5	2.3

DM= days to maturity, PH=plant height. ESH = Ethiopian local checks, entries 1 -25 are the TAMU HPD lines. *Field testing and scoring was done by EIAR.*

1. Improved TAMU lines performed similar to or better than local checks across multiple environments in Ethiopia, both under drought stress and normal rain conditions (Table 6).

2. Improved HPD/WX lines well adapted to Ethiopian environments.

B. Major challenges encountered and major project adjustments

1. *Methodology*: The **most disappointing and challenging** part of the project was quickly realizing that that the primary method we needed to identify the HPD mutation in our experimental sorghums was not reliable for the intended purpose (see Table 4). The in vitro pepsin assay is commonly used as the primary method to screen sorghums for the presence of the HPD protein body mutation; this is based on earlier reports that demonstrated that the HPD mutation caused a distinct increase in protein digestibility that could readily distinguish the HPD sorghum from the wild types. Unfortunately, we found that this assumption is only valid for HPD sorghums with a soft endosperm (like the original unimproved mutants). The *in vitro* digestibility of the improved hard endosperm lines varied widely, and appeared to be affected by various factors, including environment. Without a reliable, quick method to accurately identify the HPD nutation, our project would essentially stall.

   Through testing various alternatives, we were able to develop a new, quick, and reliable microscopy method (Field Emission-Scanning Electron Microscopy, FE-SEM) that does not require the tedious and time-consuming sample prep protocol of the current confirmatory method (transmission electron microscopy, TEM). We used the new FE-SEM method to subsequently identify the HPD trait in the experimental lines we used. Our findings in this regard are **novel** and were recently **published in a high impact peer reviewed journal** (Food Chemistry).

2. *Drought*: Severe drought in Ethiopia in 2015 significantly impacted adaptation trials of the experimental TAMU lines in Ethiopia, but also revealed an opportunity. Our initial expectation was that the TAMU lines would perform very poorly in Ethiopia. We thus decided to screen 28 lines (instead of originally proposed 4 lines) to improve our odds of identifying lines that could be hybridized into locally adapted

varieties. Surprisingly, most of the TAMU lines outperformed local Ethiopian checks under the severe drought stress. For this reason, we decided to repeat the experiment the following year, and high similar performance was revealed under non-stressed conditions. The TAMU lines were thus revealed to be highly adaptable to Ethiopian environments. They can likely directly enter the local breeding pipeline with an established hybrid seed production.

3. *Funding delays*: Because Hawassa does not have the capacity to front project funds, delayed availability of funds from USAID, especially in the final project year negatively impacted our ability to conduct a critical stakeholder project evaluation/feedback workshop in Hawassa originally scheduled for mid-2018. This workshop was meant to showcase and disseminate key project accomplishments and solicit feedback from stakeholders on the most relevant follow-up activities. The workshop has been postponed to 2019, and instead we have had to rely on phone calls with select stakeholders to strategize and plan for future activities.

4. *Gender parity in long term training*: This is an area where we have not been able to deliver on our original aspiration of having 50% female participation in graduate training (only 1 out of 4 was female). Ethiopian higher education is highly skewed towards males (more than 90% of graduate students and faculty are male), and thus finding suitable female candidates interested in graduate education has been particularly challenging. We have discussed this in depth with our Hawassa colleagues, and one strategy we propose to implement in follow up project (if successful) is to avail **graduate funding lines exclusive to female candidates**.

C. Student training achievement

1. Ms. Loza Mengistu successfully completed her MS program in 2017 and is *currently working for Castel Winery* in Ziway, Ethiopia, as a Quality Assurance Supervisor. She **has expressed interest in pursuing a PhD should our project be renewed**. She *developed the optimized sorghum injera processing method* using local technology that we have relied on throughout the project.

2. Mr. Adane Getahun has completed his research and is scheduled to defend his thesis before the end of the year. His research has been instrumental in demonstrating the performance of the sorghum lines in injera in collaboration with small scale enterprises.

3. Mr. Abadi Mezgebe completed his PhD work at University of Pretoria and is currently **back on faculty at Hawassa University as assistant professor**. Key accomplishments; *1 peer reviewed publication*, *4 presentations* at regional & International conferences.

4. Mr. Tadesse Teferra is anticipated to complete his research in the spring of 2019. He has been highly successful, winning **2 national awards** *(from American Assoc Cereal Chem Int'l)*, publishing part of his work in *the* **top-most peer reviewed research journal** in the food science field *(Food Chemistry)*, and presenting his research at 4 international conferences over the past 3 years.

5. **NOTE: Doctoral training of Hawassa University staff is especially impactful**, because at the moment, there is only one faculty member with a PhD in the entire School of Nutrition and Food Science.

D. Short-term training and outreach

Ninety-nine (99) individuals from Ethiopia (women groups, industry, small and medium enterprises, government agencies and academia) successfully trained through workshops, demonstrations, participatory product development and sensory evaluation, etc.

Results from our work disseminated through **national television, radio and and national press**.

6 - UTILIZATION OF RESEARCH OUTPUTS

A. Technologies and management practices by phase of development

1. Methods to incorporate sorghum in traditional and modern food products without negative impact on sensory quality – ***undergoing testing***.
2. Improved endosperm sorghum for protein quality and processing functionality – ***undergoing development***.

B. Intermediate outputs

Research

1. Functional superiority of HPD/WX trait in injera and batter-based products demonstrated.
2. Functional advantage of HPD trait in bread/dough system demonstrated.
3. Functional advantage of WX trait in malting documented.
4. Competitive agronomic performance of TAMU HPD/WX hybrids in Ethiopia demonstrated.
5. New rapid method to accurately detect HPD mutation in sorghum developed and successfully used.

Capacity building

1. Expanded long term research capacity at Hawassa University School of Nutrition & Food Science
2. 2 Faculty members trained, increasing PhD holders from 1 to 3 (200%).
3. 3 Food analysis and testing equipment purchased for food science laboratory use.
4. 2 MS students successfully trained.
5. 99 stakeholders trained in different areas of sorghum processing, quality, and analysis.

C. Publications

Peer reviewed publications (directly funded)

Teferra, F.T., Amoako, D, Rooney, W., Awika, J. 2019. Qualitative assessment of 'highly digestible' protein mutation in hard endosperm sorghum and its functional properties. *Food Chemistry,* 271, 561-569.

Mezgebe, A.G., Abegaz, K., Taylor, J.R.N., 2018. Relationship between waxy (high amylopectin) and high protein digestibility traits in sorghum and malting. *Journal of Cereal Science* 79, 319–327.

Peer reviewed publications (related activities)

Shreeya Ravisankar, Kebede Abegaz, Joseph Awika. 2018. Structural profile of soluble and bound phenolic compounds in teff (*Eragrostis tef*) reveals abundance of distinctly different flavones in white and brown varieties. *Food Chemistry*, 263, 265-274.

Elhassan, M.S.M., Oguntoyinbo, S.I., Taylor, J. and Taylor, J.R.N., 2018. Formation and properties of viscoelastic masses made from kafirin by a process of simple coacervation from solution in glacial acetic acid using water. *Food Chemistry* 239, 333-342.

Elhassan, M.S.M., Emmambux, M.N., Hays, D.B., Peterson, G.C. and Taylor, J.R.N. 2015. Novel biofortified sorghum lines with combined waxy (high amylopectin) starch and high protein digestibility traits: Effects on endosperm and flour properties. *Journal of Cereal Science* 65, 132-139.

Presentations at scientific conferences

1. **A total of 11 presentations** by graduate trainees; Ms. Loza Mengistu (3) Mr. Mezgebe (4) and Mr. Teferra (4) at national/international scientific conferences to disseminate research findings.

2. Mr. Teffera won 2 product development competition awards at international conferences organized by America Association of Cereal Chemists Int'l in 2016 & 2017, using sorghum as primary ingredient.

Figure 1. A collage of select project activities and output : A) Loza Mengistu (MS trainee) trains women entrepreneurs on food quality testing, B) Professor Kebede setting up a new Mixogram at Hawassa University, C) Experimental TAMU HPD sorghum at the EIAR test field in Mieso, Ethiopia, D) FE-SEM images of HPD and wild type sorghum protein body structure, E) 50% HPD sorghum injera from a small scale processor's kitchen, Hawassa, F) Bread with 10% HPD sorghum substitution from a small scale bakery, Hawassa, G) HPD-WX sorghum malt.

7 - STATEMENT ON PROPOSED FUTURE ACTIVITIES FOLLOWING PHASE I RESEARCH ACCOMPLISHMENTS

A. Statement on future activities in phase II

Our findings have generated considerable excitement among various stakeholders in Ethiopia, from farmers who saw the experimental sorghums in field trials, to food processors who witnessed processing/product performance and are asking where to source the grain, to government officials and scientists interested in establishing local seed production. We hope to capitalize on this momentum by focusing on activities that will **advance on our phase I findings and set the stage for a successful sorghum food use infrastructure**. We will primarily target three areas in collaboration with local stakeholders; pilot processing technology development, scientific research, and hybrid seed/grain production. The focus areas we will target include:

(i) Pilot sorghum processing and product technology development

Taking advantage of the improved functionality of the HPD/WX trait we demonstrated in various products, our primary aim will be to establish commercially viable sorghum food processing and products using the *novel sorghum lines*, *in tandem with well adapted locally available sorghums with similar quality attributes* for a broad market appeal in Ethiopia and larger E. Africa. An advantage we see in the Ethiopian food processing system is that it is still highly fragmented and dominated by small and medium scale enterprises. Such enterprises are generally far less risk averse, and more open to adopting new technologies that than the large corporations. Our interactions with them in phase I have been very encouraging. Four sub-objectives will be targeted for this focus area:

(a) Establish commercially viable injera processing and products using the novel sorghum lines and well adapted locally available sorghums as a supplement to teff in Ethiopia.

Experimental. In the first phase, the HPD/WX trait was found to improve injera quality and shelf-life as compared to normal sorghum lines. In fact, 50:50 blend of teff and heterowaxy-HPD sorghum produced injera that was similar to or better than control 100% teff in overall acceptability and other sensory parameters. Another advantage is that the HPD/WX lines fermented much quicker (24 h) than normal sorghum or teff (at least 48 h) which could significantly enhance process efficiency. We will focus on optimizing pre-scale up of partial teff replacement, using the conditions we established in phase I as the reference point. Usually, moving from lab scale to pilot scale requires considerable adjustments in process conditions (e.g., fermentation time/temperature), raw material particle size, blend ratios, etc. Consumer evaluation of injera will be the primary criteria for assessing product quality and market potential.

We expect to develop practical and robust sorghum-teff injera processing protocols that can be readily adapted by commercial injera processors under various conditions and scales. We also anticipate this will lead to opportunities for production of commercial *flour blends for retail consumer home use*.

(b) Establish commercially viable processing and products using the novel sorghum lines and well adapted locally available sorghums as a supplement to wheat.

Wheat imports continue to be a major economic burden to Ethiopia and many African countries. Consequently, partial replacement of wheat with the new highly functional sorghums without impacting product quality has significant economic potential in Ethiopia and beyond.

Experimental: In phase I we found that HPD trait functions differently in wheat-based products depending on product type. In breads, for instance, the HPD trait significantly improves sorghum functionality in absence of WX trait. On the other hand, in batter-based products like pancakes and cookies, the WX trait enhances the functional advantage of HPD. Furthermore, different products require different blending ratios for optimal quality. We will use the knowledge gathered from the extensive laboratory testing as foundation to develop scalable processes and blends to produce marketable wheat-based products. Several small/medium scale bakers in Hawassa have expressed strong interest in working with us on scale up process development to obtain marketable products. Developed processes will be shared with larger scale bakers in Addis Ababa and other urban regions.

'Market-ready' products developed will be showcased to wheat millers and government agencies involved in wheat import and flour milling & blending. Results from this sub-objective will have broad applicability within the larger East African region and beyond.

(c) Establish the suitability of the novel sorghum hybrids and well adapted locally available sorghums for commercial brewing in Ethiopia.

Malting and brewing in Ethiopia (and other countries in Africa) relies heavily on costly imported barley. For this reason, many companies have experimented with sorghum, and a few companies contract farmers to produce specific sorghum varieties for brewing. Most of these sorghums are not ideal for malting/brewing.

Experimental: In phase I, we demonstrated that WX trait is most important in improving sorghum functionality for malt production. On the other hand, combined HPD/WX trait was previously demonstrated to significantly enhance efficiency of ethanol production from sorghum. Thus, the HPD/WX sorghum would be ideal for *both malt production and adjunct use* in brewing, a dual benefit that we expect will be highly attractive to the brewing industry. Because commercially viable waxy sorghum germplasm is available, we will use waxy sorghum to optimize pilot scale malt production process. We will concurrently work with a local brewing company to optimize pilot scale fermentation demonstrating relative efficiency of the HPD/WX sorghum for adjunct application.

Using the improved sorghum as adjunct in brewing is especially attractive and has **high potential for adoption** since the process is straightforward and benefits are easy to demonstrate. It also does not require major process adjustments or manpower training by brewing companies. This would also provide a viable mechanism to develop production and market value chain for the improved sorghum due to a predictable and relatively large capacity market.

(d) Evaluate and establish industrial sorghum milling for use in various cereal based foods.

A primary determinant of the quality of any product made from cereal grain is how the grain is milled. Therefore, ensuring that high quality sorghum flour is available as a raw material in Ethiopia is critical.

Experimental: There is no commercial sorghum flour milling in Ethiopia, while huge quantity of the crop is hammer milled and consumed in villages in different forms of food. Therefore, a study on pilot industrial sorghum milling for use in various cereal based foods is very important. From a laboratory scale, we have demonstrated that the best and most consistent quality sorghum flour for baking applications is produced by attrition technology, as opposed to the impact milling. We thus propose to set up pilot scale pin mill and/or roller mill for sorghum flour processing and demonstration. Conditions to efficiently mill sorghum to maximize flour yield, quality, and shelf stability will be developed. This has major potential to unlock commercial sorghum use for bakery applications, and retail flour production. *Funds to purchase capital equipment will likely be needed to accomplish this objective*.

(ii) Establish improved HPD/WX sorghum seed and grain production in Ethiopia
For our work to be successful and produce intended impact, the HPD/WX sorghums must be successfully produced in Ethiopia on a commercial scale. Because we have demonstrated the competitiveness and adaptability of these lines across multiple environments in Ethiopia, we believe this task can be readily accomplished. The biggest bottleneck, perhaps, will be the viability of the local hybrid seed production infrastructure. Two key elements of this Objective will need to be accomplished concurrently:

(a) Large scale grain production for product development and demonstrations.
Significant quantities of grain will be needed on an immediate basis to execute the various tasks proposed under Objective I, and also make grain available for our industry partners for in-house testing as needed. In collaboration with EIAR, we will identify locally available sorghum varieties with the equivalent endosperm functional properties of interest. These varieties will be multiplied to produce an immediate source of grain for local process development and optimization, as well as initiating prospective end-user market linkages.

Concurrently, both TAMU and EIAR will work to multiply 3 high potential improved hybrids we have identified with the traits of interest (HPD/WX, HPD/non-WX, and WX) in Ethiopia and Texas. The grains will be used for product development testing, demonstrations, and for supplying grain to industry partners for in-house testing. The strategy will help establish prospective market linkages with the target food and brewing industry. Based on our discussion with EIAR, one sorghum producing region (**Melkassa or Mehoni**) will be targeted as grain production and 'demonstration center' to improve efficiency and market linkage potential. This strategy has been demonstrated to be viable in Ethiopia sorghum production targeting brewing industry.

(b) Hybrid seed production towards commercialization of improved sorghum hybrids in Ethiopia.
TAMU will work with EIAR to initiate hybrid seed production of the improved lines in Ethiopia with the goal of developing a long-term pipeline of the high value sorghums for targeted food applications in Ethiopia. Given the long-term nature of this undertaking, we will actively engage the National Sorghum Program, government agencies, USAID mission in Ethiopia (to identify long term synergies with in-country complementary projects), and other donors to maximize long term potential of the high value sorghum seed production in Ethiopia. **The USAID mission in Addis Ababa has been especially helpful in sharing valuable information on in-country activities and priorities.**

(iii) Establish mechanisms by which the HPD mutation impact sorghum functionality
The broader long-term impact of this project is dependent on addressing key questions raised/not addressed by our findings in phase I. One key research area that needs immediate attention is uncovering the mechanisms by which the HPD mutation impact sorghum functionality. We observed dramatic changes in sorghum starch behavior, especially the waxy starch, in presence of the HPD trait, suggesting specific interactions between these components. We believe the HPD-starch interactions are key to the improved textural properties we observed in various products.

For food product development efforts to be broadly successful and sustainable, the mechanism by which the HPD trait impacts sorghum endosperm functionality and product texture must be demonstrated. This way, the most efficient processing technologies that take advantage of the trait to maximize benefits to food quality can be devised. Furthermore, this would lead to identifying alternative uses for these sorghums to maximize benefits to sorghum producers. The effort may also enable us to explain the underlying mechanisms for the broad variability in the *in vitro* protein digestibility we observed in the

HPD germplasm.

If resources allow, **a secondary and relevant research objective** we are highly interested in, is **establishing heritability of the HPD mutation** in sorghum. Because on-going breeding efforts will be required to maintain market potential of the HPD trait, understanding its heritability will be necessary for efficient genetic improvement. With the new method we recently developed that can qualitatively and reliably establish the presence of the trait, this effort is more likely to be feasible.

B. Linkage to Phase I objectives and activities

Our proposed activities are a direct continuation on the successes of our initial objectives as outlined in Section (a) above. This phase primarily involves scaling up both product technologies and processes, as well as grain production for prospective commercialization in Ethiopia. Direct collaboration with industry partners and potential commercial end-users will be a priority on the technology side. Linkage with farmers in our initial target region will be a priority on the production side.

C. Training and outreach objectives

Medium- to long-term training
The training will focus on building immediate capacity needed to execute project tasks. We have successfully trained two highly motivated PhD faculty members (Abadi Megzebe and Tadesse Teferra), who will triple the number of PhD holding faculty members in the food science program at Hawassa from 1 to 3. We will work to equip them with the tools necessary to establish a successful and sustainable research program at Hawassa University. We intend to do this by giving them local mentorship in designing and executing research projects, proposal writing, student advising, data analysis and scientific writing, among others. We plan to also provide them with 3-6 months post-doctoral opportunities in other institutions to further their development and collaborative network. Because **poor remuneration** is a primary reason most trained personnel leave Ethiopian institutions, ability to supplement salary through external grants is critical in motivating and retaining new faculty. Successful grantsmanship will thus be an important part of the post-doctoral training of these newly trained faculty members.

We also plan to train at least 3 MS students in Ethiopia and one PhD student in the US to help in executing the project objectives. Both Abadi and Tadesse will be involved as faculty advisors of the graduate students. Female student recruitment will be a top priority.

Short-term training
Because our work will heavily involve various stakeholders, we will organize numerous workshops for engaging the stakeholders and initiate technology-transfer activities. At least one major workshop is planned per year of the project in Ethiopia to meet with women community groups engaged in food processing, small and medium scale enterprises (SMEs), farmer groups, and selected outreach NGOs and government entities. Training will be provided as necessary on food processing and product development suitable for SMEs. Feedback will be sought from these groups on how to refine research strategies to ensure the most relevant outcomes are achieved. Government representatives will be involved in organizing these workshops, and policy issues that may favorably impact sorghum value chain will be discussed.

GENETIC IMPROVEMENT OF SORGHUM FOR RESISTANCE TO FUNGAL PATHOGENS

1 – PRINCIPAL INVESTIGATOR

Tesfaye Mengiste, Plant pathologist - *Purdue University, USA*

2 – RESEARCH TEAM

Co-Investigator: Tesfaye Tesso, Sorghum breeding - *Kansas State University*

Partners: Ethiopian Institute of Agricultural Research (Melkasa, Pawe, Jimma Research Centers)
Oromia Agricultural Research Institute (Bako Research Center)
Haramaya University, Jimma University
Collaborators (USA): Gebisa Ejeta, Purdue University

3 - PROJECT GOALS AND OBJECTIVES

The overarching goal of this project is to improve the livelihood of sorghum farmers by providing technology options composed of disease resistance and well adapted sorghum varieties. Fungal diseases, sorghum anthracnose and grain mold are two of the most important diseases of sorghum in the humid intermediate altitude regions in Ethiopia. Genetic resistance is the only feasible disease control option for these two diseases and significant increases in sorghum productivity could be achieved by providing durable and broad spectrum resistance to sorghum grain mold, anthracnose and other foliar diseases. The stated project goals will be achieved through disease resistance phenotyping of extensive genetic resources, resistance breeding, supported by molecular and genomic tools for identification and characterization of genes and genomic regions underlying broad spectrum disease resistance. The spectrum of natural variation will be explored to identify disease resistance to combine with other adaptive traits to create high yielding sorghum varieties. The germplasm evaluations that make use of the unique environmental conditions of the target region will be strengthened by the power of next generation sequencing and mapping approaches to identify genes underlying quantitative traits such as grain mold which have traditionally been intractable. To guide the breeding, and enhance resistance identification, the prevalence and nature of fungal species causing grain mold and strains of anthracnose in the target area will be studied. Further, the project aims to strengthen the capacity of local research institutions, by providing graduate education in critical areas that are likely to boost the capability of next generation of breeders and plant pathologists.

Specific objectives

1. **Objective 1.** Conduct genomics and molecular analysis of the Ethiopian sorghum germplasm collection for disease resistance and other traits.

2. **Objective 2**. Develop new sorghum varieties with resistance to major diseases, improved yield potential, and broad adaptation.

3. **Objective 3.** Identify and characterize sorghum anthracnose resistant genes through genomic approaches.

4. **Objective 4.** Conduct next generation mapping using recombinant inbred lines to elucidate mechanisms of grain mold resistance and identify novel variants.

4 - OVERVIEW OF ACTIVITIES

Phase I activities spanned breeding, pathology, genetics/genomics, and molecular biology spread under the 4 main specific objectives above. The activities ranged from enabling strategic research including gene discovery, translational and applied research to achieve the major goals and objectives. Comprehensive field, greenhouse and laboratory studies were conducted to identify anthracnose and grain mold resistance germplasm with better adaption to the target regions in Ethiopia. Genetic and genomic studies were conducted to characterize resistant germplasm, define disease resistance loci, and identify resistance genes/alleles, and determine their utility in the breeding program in Ethiopia and beyond. These studies have laid the foundational knowledge, materials and tools to drive crop improvement in the target area and beyond. The fungal side of the interaction was also studied to determine the species composition of molding fungi and also variation in *Colletotrichum sublineolum,* the causal agent of sorghum anthracnose disease. In parallel, to enhance the institutional capacity of national and regional research institutions in Ethiopia, we have trained students and research staff in sorghum pathology, genomic tools and resistance breeding. We were involved in outreach of the SMIL activities at various venues in Ethiopia and the US.

5 - ACCOMPLISHMENTS

A comprehensive and multifaceted research program was initiated to achieve project goals. Progress has been made in discovery, translational research, breeding/pathology, and training of graduate students and researchers. The current discovery work will pave the way for advancing future crop improvement whereas the applied plant pathology and resistance breeding work is generating genotypes that will be released directly or resistance traits incorporated into existing elite materials. Some of these materials are at an advanced stage towards a varietal release. Besides contributions in germplasm enhancement, the prominent impact of this project is that it instigated a disease resistance breeding and plant pathology program both at the federal and regional levels. Despite decades of extensive sorghum improvement work in Ethiopia, disease resistance breeding and sorghum pathology have not been a major objective of research institutions.

Significant activities, major achievements and scientific advances:

1. Phenotyping and genomic characterization of a large collection of 2010 Ethiopian landrace sorghum accessions completed. Among these 1,425 lines were genotyped through genotyping-by-sequencing (GBS) approach.

2. A large-scale genome wide association mapping (GWAS) of Ethiopian sorghum landrace collection was conducted using the field based phenotypic data and the GBS data. GWAS identified loci and candidate genes underlying 8 different traits. Genomic and statistical analysis established a core-collection based on representation of 12 distinct genetic clusters to serve as sources of unique genes for various desirable traits.

3. Conducted disease nurseries for multiple years and locations to identify grain mold and anthracnose resistant germplasm in Ethiopia. These materials are now advanced to national variety trial (NYT), regional variety trials (RVT) and preliminary yield trials (PYT) which will ensure release of multiple varieties in Phase II.

4. Explored natural variation and identified distinct genes and/or loci representing independent mechanisms of resistance to anthracnose and/or grain mold resistance. The introgression of these resistance genes into many of the widely adapted elite local materials is at an advanced stage at EIAR (Melkasa) and Bako.

5. Identified and cloned a disease resistance gene, *ANTHRACNOSE RESISTANT GENE1 (ARG1)* that confers broad spectrum and multi-pathogen fungal resistance through next generation mapping. Molecular and genetic characterization completed.

6. Multiple lines with broad spectrum or race specific resistance were identified, their mode of inheritance determined by testing for resistance to *Colletotrichum sublineolum* strains from the US and Ethiopia. *C. sublineolum* is the causal agent of sorghum anthracnose. Three dominant and independent *ANTHRACNOSE RESISTANT GENES* (ARGs), and one recessive *ARG* locus genetically defined. Mapping and gene identification of the specific genes (ARG2-4) will be completed in the next phase.

7. The relative distribution and importance of different fungal species causing grain mold on sorghum were determined. Components of the species complex causing grain mold determined in Ethiopia.

8. The variations in *C. sublineolum* strains from Ethiopia were determined. A subset of these *C. sublineolum* strain are being used for disease resistance screening both in Ethiopia and at Purdue University.

9. Genome wide association studies identified multiple genomic regions for grain mold resistance. Among these, significant SNPs were identified in a MYB transcription factor gene which controls the biosynthesis of an antifungal secondary metabolite.

10. Developed molecular markers specific to resistance genes or loci for molecular breeding and facilitate transfer of the resistance genes into elite materials.

11. Provided training for 4 PhD and 2 MS graduate students, provided academic advice, short term training, and technical support by providing equipment and reagents.

A. Achievements by project objectives in Phase I proposal.

Objective 1. Conduct genomics and molecular analysis of the Ethiopian sorghum germplasm collection for disease resistance and other traits.

This objective is now fully and successfully completed. Ethiopia is the center of origin and diversity for sorghum, among others, and has been the source of some of the most valuable genes for improvement of the crop. A total of 2010 Ethiopian sorghum landrace accessions were sampled from more than 9000 sorghum accessions maintained at the Ethiopian Biodiversity Institute and the national agricultural research centers in Ethiopia. The selection of accessions was designed to represent different sorghum growing regions and different agro-climatic zones. True-to-type lines were isolated for these accessions and seeds amplified in the first two years of the project. Then, all the materials were planted at different locations in Ethiopia and large scale phenotypic data on many agronomic and disease resistance traits were documented for several years. The first two manuscripts describing results from this activity were recently submitted for publication. Additional highlight of our results are presented below.

1.1 A large-scale genome wide association analyses of Ethiopian sorghum landrace collection reveal loci associated with important traits.

Of the 2010 accessions, 1425 were genotyped through genotyping-by-sequencing (GBS). A total of 72,190 robust SNP markers were identified. Pairwise distance-based hierarchical clustering identified 11 distinct clusters. Consistently, principal component analysis further described population stratification. GWAS analysis based on compressed mixed linear model on 1425 sorghum accessions identified SNPs with significant association (FDR ≤ 0.05) to eight different traits. The percentage of the total phenotypic variation explained with the significant SNPs across traits ranges from about 2 to 43%. A genome-wide association study (GWAS) was initiated to define genetic loci and single nucleotide polymorphisms associated with desirable traits. Candidate genes that show significant association with different traits were identified. Interestingly, the sorghum *bHLH* transcription factor, *ABORTED MICROSPORES* is identified as a strong candidate gene for male fertility. Notably, sorghum CLAVATA1 receptor like kinase, known for regulation of plant growth, and the *ETHYLENE RESPONSIVE TRANSCRIPTION FACTOR* gene RAP2-7, which is known to suppress the transition to flowering in other plant systems were significantly associated with plant height.

> Main highlight of this work: Robust SNP markers, candidate genes and novel loci controlling various traits were identified. The genetic architecture of natural variation in Ethiopian sorghum germplasm transcending a wide-range of environmental and agro-climatic conditions is established. Our observations contribute to the characterization of genes and alleles controlling agronomic traits. Once the candidate loci associated to traits are validated, molecular markers will be developed and used in marker assisted selection for trait improvement. Finally, the availability of sequences for such a large population of Ethiopian germplasm has laid the foundation for future genetic studies in many different traits. Ultimately, the genetic variants and newly defined loci will contribute to a better understanding of the genetic mechanisms underlying these traits and improvement of the crop.

1.2. A core subset of Ethiopian sorghum germplasm established based on next-generation sequencing and phenotype diversity as a valuable genetic resource.

Understanding population genetic structure and diversity of a crop is essential in plant breeding options and designing selection strategies. Hierarchical cluster analysis and PCA, both revealed reduced genetic diversity across improved sorghum varieties including pedigree lines in the breeding pipeline from Ethiopia. Most of these varieties have been developed for dry lowland climate. Of the total 33 varieties released only two varieties are developed for wet lowland climate sorghum growing areas. The two varieties released for this region are from landrace collection released for farmers after evaluation of their performance, suggesting lack of a deliberate breeding effort for the wet lowland areas.

To further improve utilization of germplasm, a core subset was selected, from the 2010 large collection, following posteriori grouping of genotypes based genetic cluster group obtained through GBS analysis followed by stratified random sampling using quantitative traits. All genotyped accessions were classified into one of 12 groups and the remaining accessions with no genotype data were treated separately as independent missing group. All accessions were therefore classified into one of 13 groups. Stratified selection of core collections resulted in a core subset of 387 accessions, representing 20% of the collection. Shannon Weaver diversity index across the two groups (base collection and core subset) for categorical traits also confirm maximum diversity captured in the core and its representation of the entire collection. Frequency comparison based on chi-square for entire and core collection showed no significance difference ($P<0.05$). Likewise, core evaluation based on range, mean and variance comparison of the entire and core collection showed no significance for all seven quantitative traits studied. The manuscript describing this work is in preparation with a planned submission in December 2018.

> Significance of this work: The catalogue of data on agronomic traits, fertility reactions, and the GBS data are now available both at the EIAR and at Purdue. It will be made available to the wider scientific community as soon as the publication is accepted. The DOI or SRA number will be provided with the publication and reported to SMIL.
>
> Genomic analyses revealed that the Ethiopian landrace sorghums could be categorized into 11 distinct clusters based on genome wide analyses. A sub-collection of 250-300 lines can capture the entire 12 clusters of the Ethiopian sorghum germplasm without the need to screen the entire large collection. These materials harbor unique genes and alleles that confer adaptation to

the various agro-ecological zones and to various biotic and abiotic stresses. The genotypic data and the purified germplasm are genetic resources for future breeding and genetic studies in Ethiopia.

GBS data: Researchers at the Ethiopian Institute of Agricultural Research already have access to the GBS data. EIAR staff are also co-authors on multiple manuscripts resulting from this work and are aware of the process we are following to make these publically available. The data are also organized to be deposited at public repositories and at a serve in the EIAR and other local institutions. The raw and partially analyzed genotypic and phenotypic data will also be deposited at the Ethiopian Biodiversity Institute. Ideally, the data will be linked to the original germplasm entries that the institute holds.

Training and outreach about the phenotypic and genotypic data on the large and core-collection of land races and GBS data: Training will be provide to local staff on the use of GBS data and the analytical and bioinformatic tools. Without the required training, these will not have the desired impact. The training will be done early in phase II of the project. To achieve this goal, we have initiated conversations on how to deposit the data and organize the necessary high capacity storage devices on which to save the data and make it accessible. Discussions were also recently held with Haramaya university faculty and students about the SMIL project and the genotypic data on sorghum land races. The sequence data will be made available to Haramaya university faculty and students to make it accessible to students working on sorghum for their graduate research. Current and future graduate students at Haramaya and other local universities will benefit from these resource. The SMIL PIs are involved in graduate mentoring at several universities in Ethiopia and will promote the use of these data where appropriate for greater impact.

Objective 2. Develop new sorghum varieties with resistance to major diseases, improved yield and broad adaptation.

2.1. Disease resistant sorghum lines identified and incorporated into the national and regional breeding programs: Significant progress have been towards the development of disease resistant varieties. Over the last 4 years, disease nurseries were conducted at different locations in Ethiopia to identify disease resistant materials. These materials were derived from local germplasm, genetic studies at Purdue in the PIs and his collaborators laboratory. Detailed analysis of the data, both genomic and phenotypic, was conducted on the performance of the materials for disease resistance and other desirable traits. Sorghum lines that show broad spectrum resistance to foliar and grain diseases were identified. The identified resistant lines are now advanced to additional rigorous multi-location testing to select the best materials to be released as varieties in the diseases prone regions in Ethiopia.

2.1.1 Preliminary Yield Trials (PYT): From disease nurseries conducted over the years, 51 grain mold and anthracnose resistant lines are advanced to PYT towards identifying the best performing lines for potential release. A subset of the 51 resistant lines show resistance to leaf diseases (including anthracnose) and grain diseases based on multi-year and multi-location field data under natural heavy infestations. In addition, 106 local landraces and 2 introduced lines are selected for further evaluation in a different set of preliminary trial being conducted in 2018 season.

2.1.2 National Variety Trial (NVT): A total of 12 selected genotypes from the 2017 disease nursery that showed broad-spectrum resistance have been advanced in to a national variety trial stage which are being evaluated in 2018 season along with other advanced lines. Some of the candidate materials with broad-spectrum resistance are ETSL 100644, ETSL 101327, ETSL 100920, PML981475, PML981442, PML981472, PML981476, and PML981488. Rigorous selections through subsequent trials will identify the best performing materials for eventual release as varieties, targeting the low and intermediate altitude regions in Ethiopia. Final determination on specific lines to be released will be reached after repeated trails in the next 2-3 years. Genotypes with broad-spectrum diseases resistance, yield potential, and broad adaptation will be selected and released as varieties.

2.1.3. Regional Preliminary and Regional Variety Trials coordinated from Bako (also see results under section 6, utilization of research outputs). A key result with a potential impact to the target region includes 21 selected materials from this SMIL project which are now advanced to the regional variety trial stage (RVT) by Bako center. RVT is equivalent to the National Variety Trial (NVT) coordinated by EIAR. We are confident that 2-3 lines will be advanced for verification test in the coming season and at least one will be released.

2.2. Introgression of resistance genes into high yielding and adapted sorghum genotypes in Ethiopia: To transfer resistance genes into adapted and high yielding sorghum varieties/genotypes in Ethiopia, crosses were made between our resistant materials and elite and widely adapted local materials lacking resistance traits. Segregating and subsequent generations are continually tested in the field to select materials with the combination of the desired traits. The transfer of ARG1 and other loci are being carried out at the EIAR and Bako Agricultural Research Center, Oromia Agricultural Research Institute (OARI). Selection of segregating generations from crosses generated are being conducted. A total of 71 new crosses (F1 stage) and 24 back-crosses (BC1F1 stage) were generated during 2017 season while 62 F2 and 162 F4 populations are being evaluated at Jimma and Assosa sites in 2018 season. Plant pathologists and breeders are involved in these effort of selecting materials from different crosses.

2.3. Identification and characterization of genotypes with dual resistance to grain mold and leaf diseases. Ten genotypes that show dual resistance to anthracnose and grain mold were identified from a large genetic screen conducted at Purdue, and evaluated in Ethiopia. These lines are being studied at Purdue for gene discovery through next generation sequencing and comparative genomic analysis. Besides introgression of ARG1, these lines with dual resistance have been crossed to local materials at the EIAR and Bako. Crosses to local materials *Lalo*, *Gemedi*, *Chemeda* have been made at Bako. The F3 generations are being tested for foliar and grain diseases. In a separate and extensive activity, parallel crosses have been made at EIAR with many and distinct varieties that have a wider adaptation. A large set of F3 material are being evaluated during the 2018 season.

Objective 3. Identify and characterize sorghum anthracnose resistant genes through genomic approaches.

3.1. *Anthracnose Resistance Genes* and/or loci identified paving the way for scientific advances and molecular resistance breeding: Extensive genetic, genomic and molecular studies conducted at Purdue identified four non-allelic anthracnose loci in distinct sorghum genotypes. Subsequently, the genes and/or genomic regions that determine resistance were identified. Two of these loci confer broad spectrum resistance to *C. sublineolum,* while the other two confer race specific resistance to strains prevalent in Western Ethiopia. Molecular and biochemical characterization of these materials are under way.

Notably, among these, we cloned the ARG1 gene and determined how it functions in resistance. The identification of this broad spectrum disease resistance gene was a major progress for our project both scientifically and for application in crop improvement. ARG1 confers resistance to anthracnose and other foliar diseases of sorghum. *ARG1* is unique in that it is completely nested in an intron of a unique *cis*-natural antisense transcript, designated *CARRIER OF ARG1* (*CARG*). The *CARG* and *ARG1* genes are transcribed in opposite orientations and are complementary within portions of the *ARG1* and *CARG* transcripts. Susceptible genotypes express *CARG* and two alternatively spliced *ARG1* transcripts, both of which encode putative truncated proteins that lack the leucine rich repeat (LRR) domains. In resistant genotypes, loss of *CARG* transcription is associated with elevated expression of an intact allele of *ARG1*, resulting in strong and broad-spectrum resistance to fungal species with distinct virulence strategies. Our findings demonstrate a uniquely organized sorghum immune receptor, regulated by non-coding RNA that confers powerful fungal resistance against most damaging pathogens of sorghum.

Beyond ARG1, through genetic and genomic studies conducted by graduate students at Purdue University, we discovered new germplasm that confer resistance to anthracnose. Three resistance loci designated as *ANTHRACNOSE RESISTANCE GENE 2 to 4 (ARG2-ARG3)* were defined in three distinct genotypes of sorghum. These genotypes were identified based on easily distinguishable disease resistance responses during the initial screens. Mapping populations were developed by crossing these lines to the highly diseases susceptible genotype TAM428. Genetic analyses of the F1 and F2 generations suggested that ARG2 and ARG4 are non-allelic dominant resistance genes whereas the ARG3 is inherited as a recessive trait. ARG2 is now mapped to the first arm of chromosome 05 whereas ARG4 is mapped to chromosome 8.

To identify ARG2, the entire genomic sequence spanning the mapped peak was evaluated for sequence polymorphisms to determine differences between the parental genotypes. These analyses identified a candidate resistance gene representing the ARG2 locus. The candidate ARG2 gene is constitutively expressed in the resistant parent while there was no expression observed in the susceptible parent. Further investigation has revealed the presence of a stop codon in the middle of the recessive allele of the candidate *ARG2* gene. Genetic complementation test and development of near-isogenic lines are underway to generate additional data and to better characterize the resistance gene.

Studies are underway to map and clone the *ARG3* and ARG4 genes. The *C. sublineolum* strains avirulent to the recessive resistance gene (ARG3) are virulent to the broadly-known anthracnose resistant genotypes of sorghums including SC748. Resistance mediated by the recessive ARG3 allele is stable in a wide range of temperature regimes. Hence, *ARG3* may be broadly effective in different parts of the world with elevated temperatures. The fourth locus, ARG4 confers broad-spectrum and complete resistance to leaf pathogens similar to the already identified ARG1. Genetic mapping and gene identification for ARG3 and ARG4 will be completed under Phase II to determine the specific gene underlying these resistance.

Finally, these materials have been crossed to genotypes that are high yielding and adapted to the project target regions, and subsequently will be used in the selection of resistant varieties within the sorghum breeding program in Ethiopia.

3.2. Anthracnose resistant Ethiopian sorghum landraces identified by screening germplasm from Western Ethiopia: Western Ethiopia is generally a region prone to plant diseases. Germplasm from these region is hypothesized to be a good source of resistance that have evolved under heavy disease selective pressure. A total of 225 Ethiopian sorghum genotypes collected from this region were evaluated at Assosa and Bako Agricultural Research Centres under field conditions to identify sources of resistance. The area under disease progress curve was derived from percent disease severity index and calculated for each genotype. The analysis of variance indicated that the mean square due to environment, genotype and the interaction were highly significant different ($P \leq 0.01$) for all disease parameters and indicated genetic variation for resistance between the genotypes. At Bako, 34% of the genotypes and at Assosa 8% of the genotypes exhibited resistance to anthracnose. In sum, the study identified resistant genotypes 2001PWColl#034, Acc. 71552 and Acc#15 at Bako; and 2001PWColl#052, Acc.71344, 2001PWColl#025 and 2001PWColl#050 that will have important contribution in future sorghum breeding program. These lines are now part of the regional breeding program coordinated from Bako.

Objective 4. Conduct next generation mapping using recombinant inbred lines to elucidate mechanisms of grain mold resistance and identify novel variants.

4.1 Identification of sorghum grain mold resistance loci through genome wide association mapping: Genome wide association study on 1425 diverse Ethiopian sorghum landraces identified a major grain mold resistance locus containing tightly linked and sequence related MYB transcription factor genes. The locus contains YELLOW SEED1 (Y1), a likely non-functional pseudo gene (Y2), and YELLOW SEED3 (Y3). SNPs and other sequence polymorphisms that alter the Y1 and Y3 genes correlated with susceptibility to grain mold and provided a strong genetic evidence. Accordingly, the expression of both Y1 and Y3 genes in the developing grain and glumes of a widely known susceptible sorghum line, RTx430, were severely reduced but significantly increased in the resistant line, RTx2911. In addition, the expression of flavonoid biosynthesis genes such as DIHYDROFLAVONOL 4-REDUCTASE 3 (DFR3) was significantly induced in the resistant line in response to inoculation by a mixture of spores from different molding fungi while the susceptible line displayed reduced expression. The data suggest that the MYB genes especially Y3 and its tissue specific expressions determine the differential regulation of the flavonoid biosynthesis pathway genes, the synthesis of 3-Deoxyanthocynidins and ultimately responses to molding fungi. The study also indicated that resistance to grain mold may be negatively associated with grain functionality traits such as 'injera' making quality of sorghum**.** The major highlights:

1. Grain mold resistance locus containing MYB transcription factor genes identified
2. The locus regulates responses to molding fungi in the developing sorghum grain
3. The locus contains a MYB gene, *YELLOW SEED3* previously not described
4. *YELLOW SEED3* may be involved in synthesis of 3-Deoxyanthocynidin phytoalexins
5. Grain mold resistance may be negatively associated with grain functionality traits

4.2. Phenotyping of 700 early to medium maturity Ethiopian sorghum landraces for grain mold resistance and other desirable traits.
Among the 2010 Ethiopian landrace accessions, 700 lines that range from early to medium maturity were selected. These materials are tested at two locations for a second year to identify resistant lines. The long season and very late maturing materials were excluded. Late maturing long season sorghums escape grain mold rather than having resistance when exposed to infection. In Phase II, using the available GBS data, we will conduct the GWAS for grain mold resistance in this group of materials, identify *bona fide* grain mold resistance loci, and utilize for further selection. The uncoupling of maturity from resistance will be important in the long run as the trend will be to focus on early to medium maturity groups with high yield potential that display resistance. These work is part of Habte Nida's graduate research.

4.3. Species composition of molding fungi: The species composition of molding fungi were determined in locations in Western Ethiopia where grain mold disease is a significant problem. This knowledge will guide resistance gene identifications for future activities.

4.4. Genome wide gene expression profiling of sorghum to dissect factors contributing to resistance: Genomic studies were conducted to determine global changes in gene expression underlying resistance to grain mold and anthracnose diseases. First, sorghum lines with contrasting resistance to grain mold were infected with molding fungi. Tissues samples at different stages of grain development and infection stages were sampled and RNA-seq experiments conducted at Purdue genomics center. Similar experiments were conducted for anthracnose infected resistant and susceptible lines in leaf tissues. In both cases, critical differences were observed with regards to pathways, and potential genetic determinants of resistance to anthracnose and grain mold or both. A large proportion of genes involved in the biosynthesis of various secondary metabolites were differentially expressed between the resistant and susceptible genotypes. Quantitative RT-PCR results for selected genes is consistence with RNA-seq data. We also conducted small RNA profiling from anthracnose infected tissue comparing resistant and susceptible genotypes. Interestingly, novel miRNAs with a potential role in the regulation of anthracnose disease resistance and miRNA target genes were discovered. These data will be validated and used genetic studies and crop improvement studies Phase II. The manuscript describing this work is also being finalized.

B. Major challenges encountered and resulting project adjustments

Security issues in 2017 in Ethiopia prevented travel in some regions. This security issue has now improved significantly. The involvement of dedicated local staff at the various locations helped us overcome the data collection and field evaluations at critical times. Leadership staff attrition and high turnover of technical staff are a continuous challenge. Fortunately, the target region of this project in the west was less affected. We trained staff from this regions at MS level and they are still committed and highly involved in the project. Also, although the Ethiopian partners receive their funds from SMIL, they have no ability to acquire reagents and equipment. The PIs lab spent funds allocated to his program to address those issues which was an additional burden.

C. Student training achievements, capacity building

The project achieved its objective in training of participants to improve the capacity of local institutions, by providing graduate education in breeding, genetics and plant pathology. The project provided graduate education opportunities for 4 PhD students and 2 MS level students. The project also provided a short term training visit by an EIAR staff at Purdue. The project enhanced research capabilities by providing short term training through field visits. We provided extensive germplasm and populations to local institutions. The project purchased and provided a fluorimeter and required reagents for quantification of mycotoxins.

PhD students & project

Nida, Habte. 2020 (expected graduation). Genome wide association mapping for mold resistance in sorghum. Major Professor: Tesfaye Mengiste, Department of Botany & Plant Pathology, Purdue University. Full support on SMIL 50% each T. Mengiste and G. Ejeta.

Demeke M. Bayable (expected graduation 2019). Identification and characterization of genes regulating broad spectrum resistance to anthracnose. Major advisor: Tesfaye Mengiste Department of Botany and Plant pathology, Purdue University. Full support on SMIL, Mengiste.

Xiaochen Xu (Spring Graduation, 2019). Identification and characterization of gene (s) associated with anthracnose resistance in P9830 sorghum. Advisor: Gebisa Ejeta, and co-advisor, Tesfaye Mengiste. Purdue University. Full support on SMIL 50% each G. Ejeta & T. Mengiste.

Diriba Hika (expected graduation 2020). Genetic analysis of sorghum resistance to leaf anthracnose and grain mold diseases. Advisor: Tesfaye Tesso, Kansas State University. Full support, co-PI Tesso.

Post-doctoral researcher

Gezahegn Tessema: Genomics of Ethiopian land races of sorghum for post-doctoral mentoring.

MS Students & Project

Kebede Dessalegn Lemu. Graduated 2016. Evaluation of selected Ethiopian sorghum landraces for resistance against anthracnose. MS Thesis. Haramaya University, Ethiopia. Co-advisor, Tesfaye Mengiste. Current position: Oromia Agricultural Research Institute (OARI)

Chemeda Birhanu. 2017. Genetic variability for grain mold resistance and yield and yield related traits among selected sorghum accessions in Ethiopia. MSc. Thesis. Jimma University. Co-advisor, Tesfaye Tesso. Current position: Oromia Agricultural Research Institute (OARI)

D. Short term training and outreach

Moges Mekonen: Title of training, Molecular techniques in plant pathology and detection of mycotoxins. Department of Botany and Plant pathology. November 2016. Purdue University, West Lafayette. Current position: EIAR

Training on disease phenotyping: Getachew Ayana (senior plant pathologist, EIAR), and the PI of the current project, Tesfaye Mengiste, and Moges Mekonen (Plant pathologist, EIAR) have been providing disease evaluation trainings at Jimma and Bako Agricultural research centers during the last three cropping seasons. Disease nurseries and other experiments planted at these sites were used as training grounds to train participants in disease evaluation methods. Local staff were trained in scoring and identification of the different sorghum diseases mainly anthracnose, rusts and grain mold and how to consistently evaluate the materials.

6 - UTILIZATION OF RESEARCH OUTPUTS

A. Technologies and management practices by Phase of development

Key outputs are improved sorghum genotypes that are resistant to diseases, highlighted in preceding sections. Most of our materials are being extensively used in the breeding program at the national and regional levels. Materials in the national and regional variety trails and preliminary yield trails are in their final phases of varietal development. Current evaluations suggest that 2-3 lines will be advanced for verification test next year and at least one will be released from this trial from Bako center. EIAR is conducting national variety trials and we expect 1-2 lines will be selected for a final verification.

In addition, molecular markers for breeding, extensive sequence information, and basic knowledge on pathogen strains and species were generated which will be leveraged in the future. Scientific advances that could be directly used include the ARG1 gene and how it is uniquely regulated by a natural antisense RNA, novel disease resistance genes, and how these can be used in biotechnological approaches including CRISPR.

B. Intermediate Outputs

This project was a boost to the national and regional research programs with some materials already reaching the national variety trail stage (see section 6A above, and objective 2 achievements). There was no prior support or initiative on a pathology centered research program. This project catalyzed a series of studies that focused attention to crop disease problems, and an array of out puts generated that are now part of the EAIR and regional programs. Resistant germplasm, segregating populations, know-how, and tools generated support the local Ethiopian institutions. Our results will significantly contribute to the overall mission of USAID, SMIL and the local institutions towards improving food security for small holder farmers. Multiple manuscripts are being jointly published with local researchers in Ethiopia which also contributes to staff development (see page 11). Additional details are highlighted below.

1. The materials from this project have entered national and regional breeding programs with a focus on foliar and grain diseases. A subset are in PYT, NVT, and RVTs, final phases in varietal development program.

2. Selection of segregating generations is underway for anthracnose and grain mold resistance. These populations are from crosses made at Bako between local materials (*Lalo*, *Gemedi*, *Chemeda*) and materials from this project. A separate and extensive crossing program has been conducted by EIAR by crossing our resistant materials to elite materials (*Dano, AL-70, Adukara, ETS 2752, 07MW6085, Assosa-1, Gambella 1107, Melkam Bobe red, Bobe white*) and segregating populations are currently being selected.

3. The SMIL materials carry desirable traits such as early maturity, stay green, anthracnose and grain mold resistance as well as high yield potential.

4. Sorghum regional preliminary yield trial and regional variety trial are underway at Bako and Assosa. The 36 lines included here are selected based on MS thesis of Chemeda Berhanu (SMIL sponsored) and the materials are generated from this project.

5. Bako Research center is planning to cross 17 genotypes including locally preferred landraces with Purdue's materials (PML981442, PML981369, PML981299, PML981446 and PML981570) for anthracnose and grain mold resistance. These Purdue materials were selected from our disease nurseries.

6. Besides PYT, two trials are now in progress for variety development targeting the western region. These are all based on materials selected from SMIL large collection and Kebede's thesis (SMIL sponsored). These are regional variety trial medium maturing group; and regional variety trial early maturing group.

7. Publications, presentations, posters from this project are below.

C. Publications

Fuad Abduselam, Tamado Tana, Jamal Abdulahi, Habte Nida, Taye Tadese. 2017. Evaluation of Double Cropping System for Sorghum Production at Fedis, Eastern Ethiopia. J. of Plant Sciences 5(2): 75-81

Gezahegn Girma, Habte Nida, Amare Seyoum2, Moges Mekonen, Amare Nega, Adane Gebreyohannes, Getachew Ayana2, Taye Taddese, Dagnachew Lule, Kebede Desalegn, Firew Mekbib, Ketema Belete, Tesfaye Tesso, Gebisa Ejeta, Tesfaye Mengiste. A Large-Scale Genome Wide Association 1 Analyses of Ethiopian Sorghum Landrace Collection Reveal Loci Associated with Important Traits. *(Submitted).*

Habte Nida, Gezahegn Girma, Amare Seyoum, Kebede Desalgene, Chemeda Berhanu, Taye Taddese, Getachew Ayana, Dagnachew Lule, Tesfaye Tesso, Gebisa Ejeta, Tesfaye Mengiste. 2017. Genome-wide association studies for sorghum grain mold resistance. (submitted).

Fuyou Fu, Sanghun Lee, Chao-Jan Liao, Demeke M. Bayable, Adedayo Adeyanju, Damon Lisch, Gebisa Ejeta, and Tesfaye Mengiste. 2018. Broad spectrum and complete fungal resistance is conferred by a natural antisense regulated immune receptor in sorghum. (submitted)

Fuyou Fu, Sanghun Lee, Chao-Jan Liao, and Tesfaye Mengiste. Genome-wide micro RNA and gene expression profiling uncovers processes underlying resistance and susceptibility in sorghum. (In preparation, submission Nov 2018)

Gezahegn Girma, Habte Nida, Amare Seyoum, Kebede Desalgene, Chemeda Berhanu, Getachew Ayana, Tesfaye Tesso, Tesfaye Mengiste and Gebisa Ejeta. 2018. Next-generation sequencing and phenotype based diversity analysis and core subset development in Ethiopian sorghum germplasm collection. (In preparation, submission Dec., 2018)

Habte Nida, Sanghun Lee and Tesfaye Mengiste. Differential regulation of secondary metabolites genes correlated with resistance to grain mold in the developing grains of sorghum (In preparation, Planned publication 2019).

Adeyanju, A., T. Mengiste and G. Ejeta. 2018. Inheritance and molecular characterization of an anthracnose resistance gene in sorghum line SRN39. (In preparation, 2019 publication).

Conference presentations and abstracts

Tesfaye Mengiste, Sanghun Lee Fuyou Fu. Sorghum pathology with a focus on genetic disease resistance mechanisms. University of Cambridge, England, August 18-19, 2018.

Tesfaye Mengiste, Sanghun Lee Fuyou Fu. Application of biotechnology for Crop improvement. Sorghum pathology with a focus on genetic disease. Haramaya University, African Center of Excellent for Climate smart Agriculture. Haramaya, Ethiopia, August 21-24, 2018.

Demeke Bayable and Tesfaye Mengiste. 2018. Identification and Characterization of Host Resistance Genes and Mechanisms to Sorghum Anthracnose. Sorghum in the 21st Century. Cape Town South Africa. 9-12 April, 2018.

Habte Nida, Gezhagen Tessema, Gebisa Ejeta, Tesfaye Mengiste. Identification of Sorghum Grain Mold Resistance Loci through GWAS. Sorghum in the 21st Century. Cape Town South Africa. 9-12 April, 2018.

Gezahegen Tessema, Habte Nida, Tesfaye Tesso, Gebisa Ejeta, Tesfaye Mengiste. Sorghum Genetic Diversity Study and Gene Mining for Important Traits. Sorghum in the 21st Century. Cape Town South Africa. 9-12 April, 2018.

Fu F, Ejeta G, Mengiste T**.** 2016. Identification of broad spectrum anthracnose resistance QTL in sorghum by whole genome sequencing. Plant and Animal Genome Conference (PAGXXIV). January 9-13, 2016, San Diego, CA, USA.

Mengiste T. 2017. Mechanisms of resistance to biotrophic and necrotrophic fungal pathogens causing diseases in sorghum. Gyeongsang National University. August 10, 2017.

Mengiste T. 2017. Broad spectrum and complete fungal resistance is conferred by a natural antisense regulated immune receptor in sorghum. 1st Konkuk University Plant Science Symposium. Seoul, Korea. August 8, 2017.

Mengiste T. (2014). Induced resistance to post harvest plant pathogens. *In* Induced resistance to Plant pathogens. Schwan-Estrada, KR, Mendes da Silva C, Collella JCT, (Eds) Proceedings of the conference on induced resistance, Maringa, Brazil, November 19-21. Pp 272

Mengiste T. 2014. Application of translational research for crop improvement. Ethiopian Institute of Agricultural Research, Ethiopia. October 24, 2014.

Mengiste T. 2014. Regulation of plant immunity to fungal pathogens, BASF Plant Science, Research Triangle Park, North Carolina. Nov 10, 2014.

Mengiste T. 2014. Plant immunity to fungal pathogens, Michigan State University. Plant, Soil and Microbial Sciences Seminar Speaker, October 9, 2014. East Lansing,

Mengiste T. 2015 Mechanism of fungal resistance in Crop plants. April 14, 2015, University of Wisconsin, Department of Plant Pathology, Madison.

Recent Poster Presentations

Chemeda Birhanu, Sentayehu Alamerew, Dagnachew Lule, Tesfaye Tesso. Genetic variability and associated traits for grain mold resistance among sorghum accessions in Ethiopia. Sorghum in the 21st Century. Cape Town South Africa. 9-12 April, 2018.

Kebede Dessalegn, Firew Mekbib, Tesfaye Mengiste. Evaluation of selected Ethiopian sorghum genotypes for resistance against anthracnose. Sorghum in the 21st Century. Cape Town South Africa. 9-12 April, 2018.

Moges Yirsaw, Tesfaye Mengiste, Getachew Ayana, Taye Mendaye. Exploiting the untapped genetic variability of Ethiopian sorghum genotypes for resistance breeding for anthracnose *Colletotrichum sublineolum*. Sorghum in the 21st Century. Cape Town South Africa. 9-12 April, 2018.

Xiaochen Xu, Tesfaye Mengiste, Gebisa Ejeta. Identification and mapping of anthracnose resistance genes in sorghum. Sorghum in the 21st Century. Cape Town South Africa. 9-12 April, 2018.

MS Thesis:

Kebede Dessalegn Lemu. 2017. Evaluation of selected Ethiopian sorghum landraces for resistance against anthracnose. MS Thesis. Haramaya University, Ethopia.

Chemeda Birhanu. 2017. Genetic variability for grain mold resistance and yield and yield related traits among selected sorghum accessions in Ethiopia. MS. Thesis. Jimma University, Ethiopia

7. STATEMENT ON PROPOSED FUTURE ACTIVITIES FOLLOWING PHASE I RESEARCH ACCOMPLISHMENTS

A. Statement on Phase II activities

Overview: Fungal diseases, anthracnose and grain mold result in significant loss of yield, deterioration of grain quality, and are obstacles to growing high yielding varieties with shorter growth durations. Genetic resistance to grain mold and anthracnose will be a significant accomplishment in sorghum improvement Ethiopia and beyond. It will contribute to food security in the target region by reducing the impacts of major sorghum diseases. Phase II will build on Phase I discoveries, identified disease resistant sorghum variants, breeding lines, experience and network of collaborators to achieve the overall objectives conceptualized in Phase I. We laid a strong foundation for progress in Phase II (see report section). The primary activities in Phase II will focus on further developing and releasing improved varieties, gene discovery through genetic and genomics studies of materials and populations developed, characterization of a representative sample of the core collection covering distinct clusters as a source of resistance genes, and capacity building.

First, the project seeks to deliver adapted disease resistant sorghum germplasm with high yield potential. Resistant material identified through a field based multi-year and multi-location evaluations in phase I will be developed into a product in the form of improved varieties. We identified/developed sorghum germplasm including landraces, breeding lines, recombinant inbred lines, and other populations that carry unique genes/alleles that confer broad spectrum resistance to foliar and grain disease. Efforts are underway to advance some of these promising materials for eventual release as varieties. This project advanced materials into the national and regional variety trials which are expected to release varieties in the next 2-3 years. A parallel effort to incorporate disease resistance genes into widely adapted elite materials that are deficient in diseases resistance genes is underway at the regional and national levels. Thus, the breeding effort will be completed by direct release of improved materials or transfer of genes into regionally or nationally adapted materials in collaboration with EIAR and OARI (primarily Bako research center). Additional traits will be integrated in partnership with EIAR, regional institutes and Purdue collaborators. In parallel, molecular and genomic studies will be continued to identify genomic regions, genes and develop molecular marker to expedite the improvement of the crop. We will continue to leverage recent advances in genetic and genomic technologies to identify broad-spectrum resistance genes by exploring unique populations and genetic resources we developed in phase I. The project will combine a balance of strategic and discovery research with translational and applied research in pathology and crop improvement. Finally, we will continue to train staff in national and regional institutes in plant pathology, resistance breeding and molecular techniques, and provide technical support.

Specific objectives

1. Develop new sorghum varieties with resistance to major diseases, improved yield and broad adaptation by advancing materials from Phase I.

2. Identify sorghum multi-pathogen resistant genes defined by the series of anthracnose resistance loci.

3. Genetic and genomic dissection of grain mold resistance.

4. Conduct genomic, and molecular-genetic analysis of a newly established subset of the large collection of Ethiopian sorghum landraces for disease resistance and other traits.

Objective 1. Develop new sorghum varieties with resistance to major diseases, improved yield and broad adaptation by advancing materials from Phase I.

Overview: The goal is to release sorghum varieties(s) by advancing sorghum genotypes with broad-spectrum diseases resistance. This will accomplished through a direct release of the best performing and disease resistant materials currently at the final stages in PYTs, NYTs and RVTs. In parallel, the introgression of resistance genes into elite materials will be completed.

Activity 1.1 Conduct preliminary yield trails, national variety trails, and regional variety trials by advancing materials selected from disease nurseries.

We will promote both the national and regional efforts to expedite varietal development and release. Sorghum genotypes and lines that show broad-spectrum diseases resistance, broad adaptation, and yield potential were identified. These materials have entered the preliminary yield trial, national variety trial, and regional variety trial stages and are being evaluated in the current cropping season. Subsequent trials will lay the foundation for rigorous selection of best performing materials for eventual release as varieties, primarily targeting the low and intermediate altitude regions in Ethiopia. The final determination on specific lines will be reached after repeated trails in the next 2-3 years including on-farm and on-station verification trial, then a variety release committee will make the final decision.

Deliverables: 2-3 best performing improved varieties nationally and regionally.

Activity 1.2 Incorporate novel broad-spectrum disease resistance genes into high yielding and locally adapted materials.
We defined four *ANTHRACNOSE RESISTANCE GENES* (*ARGs*) or loci that confer broad-spectrum resistance. Then, we initiated crosses of these to high yielding, adapted and varieties that lack disease resistance. EIAR and OARI research staff at Bako, generated segregating populations and F3 families. The goal here is to enrich the adapted and locally preferred varieties with disease resistance traits. In phase II, these populations from these crosses will be tested in subsequent generations and lines that combine disease resistance and other desirable traits from the receiver genotype will be identified and developed into varieties. Significantly, for some of these resistance genes, we generated molecular markers to expedite the identification of recombinant genotypes. This will help initiate and strengthen a marker assisted selection program to expedite the introgression of specific genes.

Deliverables: (1) Selection of lines from segregating populations to be developed into varieties, and (2) Lines with *ARG* loci incorporated; marker development and validation.

Activity 1.3 Combine disease resistance with tolerance to acid soils
Many sorghum growing regions are challenged by fungal diseases and the parasitic weed *Striga*. We propose to stack *Striga* and disease resistance into adapted varieties. In addition, acid soils with high aluminum toxicity are major problems in Western Ethiopia. This is the same region where fungal diseases are major challenges. In our discussion with the Ethiopian collaborators, we agreed that genotypes that combine tolerance to acid soils and disease resistance traits will be important. Sorghum genotypes SC549, SC175, SC283, and SC566 all have better aluminum tolerance conferred by a major gene. We propose to stack aluminium tolerance with disease resistance into varieties adapted to the target region. The availability of the molecular markers expedites the identification of genotypes that combine these important traits.

Deliverable: Sorghum lines/varieties that combine disease and *Striga* resistance, as well as aluminum tolerance.

Objective 2. Identify sorghum multi-pathogen resistant genes defined by the series of anthracnose resistance loci.

Overview: We seek to determine the specific genes underlying broad-spectrum resistance loci in novel variants identified in Phase I. The desire to purse these studies is motivated by the potential impact on our ability to stack resistance genes with distinct resistance mechanisms, protect against broad range of strains, and accelerate breeding through molecular markers. Importantly, knowledge of genes will enable the application of technologies such as CRISPR.

Activities: This aim will have two main activities. First, the genetic resource composed of new variant genotypes, and populations that harbor broad-spectrum and multi-pathogen disease resistance genes will be provided to EIAR and Bako staff and field trials initiated. Second, we have identified and genetically defined four independent loci for resistance to sorghum anthracnose. ARG2, ARG4 define resistance loci controlled by dominant genes in the sorghum genome whereas ARG3 is defined by a receive mutation. Interestingly, similar to ARG1, ARG4 confers resistance to anthracnose and other foliar pathogens. We will identify the genes underlying these loci through next generation mapping. These materials were also crossed to preferred local materials. Once we know the specific genes we will design molecular markers.

Main activities: (1) Identify sorghum *ARG2-ARG4* genes, and determine their function; Evaluate stability of resistance under various environments, and temperature regimes, (2) Cloning and molecular characterization of these genes, and develop molecular markers, (3) RNA-seq analyses of at least a couple of these to decipher how they affect resistance, and (4) Evaluate the potential to use CRISPR for ARG3 which is a recessively inherited resistance.

Deliverables: (1) Markers for selecting populations and train people to initiate a marker assisted selection program, and capacity building to implement this approach, (2) Develop molecular markers for distinct mechanisms of anthracnose and mold resistance to enable stacking of genes for multiple mechanisms, (3) Clone at least three novel genes that confer resistance to anthracnose, (4) Determine host resistance mechanisms towards designing effective and durable disease resistance, (5) Disease resistant populations for testing in Ethiopia for eventual breeding.

Objective 3. Genetic and genomic dissection of grain mold resistance.

Overview: Grain mold resistance is multigenic, thus, full resistance requires many genes that control different resistance mechanisms. Breeding for resistance to grain mold is often a challenge due to the complex inheritance, large environmental effect and the necrotrophic nature of the grain mold pathogens. Necrotrophic pathogens kill plant cells and grow on dead and dying tissue in contrast to biotrophic pathogens. The work proposed here will lay the foundation for the long term mining of the genetic diversity based on isolated resistance genes or applying biotechnological approaches to improve grain mold diseases. First, genotypes displaying dual resistance to leaf and grain diseases will be characterized and the genetic control of dual resistance determined. Second, a subset of the core collection that is evaluated in the target region in Ethiopia will be subjected to association mapping to identify markers that are linked to grain mold resistance. These is predicted to facilitate fine mapping and the isolation of genes underlying quantitative variation. We will assemble contrasting genotypes including the most susceptible and the most resistant lines for each trait and validate the identified loci. Ultimately, genetic dissection of leaf and grain disease will be critical to combine both disease resistance traits and understand regulatory factors.

Activity 3.1 Genetic and genomic analyses of sorghum genotypes with dual resistance to leaf and grain diseases. Among the advances made in Phase I are the identification of 10 genotypes that display dual resistance to grain and leaf diseases. These materials were identified through studies at Purdue and field evaluations at Bako and Jimma. Indeed, these materials have entered the breeding programs in the EIAR and OARI for release nationally and are included in the national and regional variety trials. To add value to the on-going effort and for us to make significant scientific advances, we have initiated genetic and genomic studies to further characterize these genotypes, identify the resistance genes and determine molecular mechanisms. Among other things, it is unclear whether resistance in these materials to grain and leaf diseases are controlled by the same genes or distinct genes in these materials. Answers to this questions will determine the breeding strategy to incorporate these genes into local materials. We also do not know how many independent loci are represented in these valuable germplasm. Such knowledge will allow applications of modern biotechnology approaches such as CRISPR, and also for the genes to be useful in other regions in Africa and in the US. Knowledge of the genes provides a powerful tool to mine germplasm adapted to other regions. At Purdue, we have crossed these materials to a generally susceptible genotypes that shows DNA polymorphism with the resistant lines. The resulting population will be used to conduct next generation mapping for gene discovery, and other genetic and molecular studies.

Activity 3.2. Characterize loci identified through genome wide association studies conducted for grain mold resistance on Ethiopian sorghum landraces.
Research by graduate student Habte Nida is focusing on grain mold resistance and its genetic control. This aim will expand on his preliminary results with the main emphasis being the validation of resistance loci, gene discovery and utilization in breeding. The primary activities are to (1) Identify genomic regions and SNPs with significant impact on grain mold resistance, (2) Validate the most important determinants of resistance by using a small number of genetic variants with contrasting mold resistance, (3) Design molecular markers for the genomic region and/or genes/SNPs to facilitate selection, (4) Implement this markers in real selection processes for resistance, (5) Complete the transcriptome and metabolic profiling of the developing sorghum grain infected with grain mold fungi.

Significance: Quantitative traits such as grain mold resistance will greatly benefit from the application of molecular tools relative to monogenic traits such as anthracnose resistance. Thus, it is critical to identify loci and genes with significant impact and use it to guide selection. One such significant locus was identified recently. This locus controls the accumulation of an antifungal secondary metabolite in the developing grain tissue. The manuscript describing this work was submitted recently and was also presented at the SMIL organized conference in South Africa.

Deliverables. (1) Generate new populations for mold and anthracnose resistance, (2) Select a subset of germplasm that is early maturing and mold resistant to identify real mold resistance genes, (3) Identify disease resistance sorghum germplasm and disease resistance genes, (4) Identify genomic regions and move these into sorghum varieties that are better yielding and are locally adapted, and (5) Molecular markers.

Objective 4. Genomic and genetic analysis of the newly established subset of the large collection of Ethiopian sorghum landraces for disease resistance and other traits.

Overview: We completed extensive phenotypic characterization of the large collection of Ethiopian sorghums landrace. This was followed by genotypic analyses of the 1425 lines which allowed us conduct mapping of loci for disease resistance and 8 other traits. Analysis on pairwise distance-based hierarchical clustering identified distinct cluster groups. Subsequently a core subset was defined following posteriori grouping of genotypes based genetic cluster group obtained through GBS analysis followed by stratified random sampling using quantitative traits. All accessions were therefore classified into one of 12 groups. Stratified selection of core collections resulted in a core subset of 387 accessions, representing 20% of the collection (see reports section 1.2 for details). This systematically selected representative subset of the large collection would capture the entire genetic diversity represented in the 2010 lines. These unique gene pool covering the spectrum of natural variation will be an excellent source of genes and traits without the need to deal with the large collection (manuscript in preparation).

Activity 4.1. Detailed analyses of 250-380 lines representing genetically distinct sorghum clusters of the large Ethiopian landrace collection. While this subset is representative for all traits, in this project under phase II, the goal is to focus on foliar and grain disease resistance traits. The lines will be catalogued for future sources of resistance and detailed and accurate plant reposes will be documented. Detailed whole genome analyses will be conducted on limited and selected lines. Using locally recruited students, recombinant inbred lines will be generated for 1-2 lines with the desired traits.

Activity 4. 2. Validate GWAS data using a subset of the core-collection, identify genes and convert to molecular markers: A subset of selected and well characterized lines will be used as a source of traits and to generate recombinant inbred lines. In addition, this activity will establish a well characterized collection of sorghum lines as vital genetic resources for fungal resistance and other traits.

Resistance loci defined previously on the large collection will be fine mapped using selected lines with contrasting diseases responses.

B. Linkage to Phase I objectives and activities

The activities and objectives described for Phase II are derived from the activities in Phase I. Advances and materials from Phase I will be used as a spring-board to achieve the overall goal the specific objectives in Phase II. As an extension of Phase I, the current project is designed to release promote the disease resistance sorghum germplasm with high yield potential, wide adaption, and release varieties or incorporate resistance genes into elite materials. The sorghum variants and other germplasm defined in Phase I will be used to identify genes and alleles underlying disease resistance.

C. Training and outreach objectives

The effort here is on human and institutional capacity building by providing knowledge enhancers, short term technical and graduate training. We focus on providing training in molecular tools, plant pathology and disease resistance breeding. This project served as a catalyst to initiate a program where disease resistance is an important objectives of the breeding program to contribute to a comprehensive improvement of the commodity. Sustainability of such an effort requires a cadre of scientists with technical expertise and required skill sets. Thus, the project aims to enhance the capacity of local research institutions, by providing graduate education in critical area that are likely to boost the capability of next generation of breeders and plant pathologists.

Activities for training and outreach: (1) Enhance expertise in sorghum pathology, (2) Data transfer and training in genomic analyses, (3) Provide training and knowledge enhancers on the use of the GBS data and GWAS, (4) Molecular marker development, design and applications, (5) Continue to provide advice to students from Haramaya and Jimma Universities, (6) Provide guidance and technical support to EIAR and regional staff, (7) Graduate PhD students supported by SMIL Phase I, (8) Recruit MS student in local universities and design projects using the GBS data, (9) Sandwich program for PhD students, (10) Hands on training on disease identification, diagnosis, and disease evaluations under field conditions, (11) Field, greenhouse and laboratory based diseases assay methods

Intent to expand impact of our research beyond Ethiopia: Significant advances were made in this project. Our findings can benefit sorghum growing regions in the US and elsewhere where grain mold and anthracnose are persistent challenges. The tools we generated could be applied directly or mine germplasms around the globe for desirable alleles in the appropriate genetic backgrounds. Our results could be applied for generating fungal resistance in biomass and grain sorghums in the US and improve grain sorghum in Western Africa. With regards to feed the future goals, and SMIL activities, the PI will be excited to participate as a co-principal investigator in any plant pathology, and sorghum disease resistance initiative in West Africa. Training of students, transfer of know-how and crop improvement will be my priority rather than project management.

GENETIC ENHANCEMENT OF SORGHUM TO PROMOTE COMMERCIAL SEED SUPPLY AND GRAIN MARKET DEVELOPMENT

1 – PRINCIPAL INVESTIGATOR

Gebisa Ejeta, Professor of Plant Breeding and Genetics - *Purdue University, USA*

2 – RESEARCH TEAM

Co-Investigators: Alemu Tirfessa, Sorghum Breeder - *Ethiopian Institute of Agricultural Research, Ethiopia*
Solomon Assefa, Sorghum Breeder - *Amhara Regional Program, Ethiopia*
Eyasu Abraha, Director - *Tigray Regional Program, Ethiopia*
Assefa Taha, Director - *Oromia Regional Program, Ethiopia*
Ketema Belete, Sorghum Breeder - *Haramaya University, Ethiopia*
Temam Hussein, Plant Pathologist - *Haramaya University, Ethiopia*
Firew Mekbib, Plant Breeder - *Haramaya University, Ethiopia*
Tesfaye Mengiste, Molecular Plant Pathologist - *Purdue University, USA*
Tesfaye Tesso, Sorghum Breeder - *Kansas State University, USA*

3 - PROJECT GOALS & OBJECTIVES:

The project sought to develop a functional sorghum breeding program in Ethiopia focused on the development of adapted, high yielding sorghum hybrid cultivars for broad societal impact. The objectives of this project were to:

1. Develop and characterize a core-set of Ethiopian sorghum landrace germplasm as foundation for accelerating a sorghum breeding program for drought tolerance and resistance to the parasitic weed, *Striga*

2. Initiate a marker assisted selection program for drought tolerance and *Striga* resistance in Ethiopian sorghum landraces

3. Establish a functional hybrid sorghum breeding program to catalyze the emergence of a commercial sorghum seed enterprise system in Ethiopia

4 - OVERVIEW OF ACTIVITIES

Ethiopian sorghums have been a great source of novel genes and valuable traits for improving the sorghum crop worldwide. Sorghum is believed to have originated in Ethiopia as evidenced by the early history of domestication of the crop there. The rich diversity among the cultivated landrace varieties is readily apparent. The vast genetic variability of sorghum is credited to the wide range of the ecological habitat in which the crop evolved and the long history of human selection efforts on the crop in this ancient nation. Sorghum in Ethiopia owes its endowments to this early history of the crop's beginning. The diversity of sorghum has been further enriched by its movement to different regions of the country and beyond, resulting in distinct forms of sorghum throughout the world. Modern sorghum breeders have heavily relied on the natural diversity in sorghum landraces in search of useful traits in advancing sorghum as a feed crop in major economies, particularly in the Americas and Australia. Unfortunately, sorghum improvement in Africa lags far behind the successes that the crop has enjoyed in these other geographies. It is possible that modern research advances made on sorghum improvement in these advanced economies may potentially benefit current and future sorghum research efforts in Africa.

As in many places of the developing world, sorghum in Ethiopia is primarily grown in semi-arid regions with erratic rainfall and marginal soil quality and fertility. Sorghum yields in Africa are generally low. However, the crop is relatively easy to grow and hardy being exquisitely adapted to the conditions of the dry lands. Its tolerance to the many production constraints make sorghum the crop of choice, and a staff of life for the millions of poor smallholder African farmers who rely on it for food and livelihood. Sorghum is tolerant to common stresses in crop production. Stress tolerance in crops is a relative term, however. Stresses caused by drought and the parasitic weed *Striga* remain formidable production constraints even for sorghum in the semi-arid tropics. The preponderance of natural genetic variability for these traits among local landraces of sorghum in Africa provides great opportunities to improve the crop. Sorghums in Ethiopia are particularly endowed because of their adaptation to water stress and co-evolution with pests and disease in this ancient nation. This project attempted to exploit native genetic variation for drought and *Striga* resistance among Ethiopian landraces to develop sorghum cultivars with resistance to these important stresses.

We employed tools of biotechnology, breeding, and agronomy to unleash the potential of the crop for needy farmers. With low cost high throughput genome sequencing and bioinformatics tools available, opportunities for harnessing allelic variation in raw crop germplasm have greatly increased. We worked as members of a team in developing a core-set of sorghum germplasm upon which we sought to discover the genetic basis of traits impacting drought tolerance, *Striga* resistance, nutritional quality and disease resistance through large scale high throughput genotyping. We planned to phenotype members of this core-set for valuable traits under target environments, and through proper bioinformatics and statistical tools to identify useful allelic variations for drought and *Striga* resistance. We supported the national program, developed its local capacity, restoring rigor and discipline to the Ethiopian sorghum breeding program to produce superior cultivars that meet the needs of their climate vulnerable farmers. We worked with agronomist and economists to develop a package of genetic and crop management practices to mediate stresses and optimize yield.

The project aimed to develop a functional sorghum breeding program in Ethiopia focused on the development of adapted, high yielding sorghum hybrid cultivars for broad societal impact. We promoted the use of hybrid cultivars to strengthen the seed supply value chain and catalyze the development of a commercial sorghum seed enterprise system in the country. The aspiration of building a commercial value chain system for sorghum in Africa is among the most badly needed investments in Africa. This project was a modest investment towards that goal through strengthening of the program in sorghum research, and these goals are loftier than what the project was fully able to achieve. We believe, however, that he project did contribute to the ongoing broader engagement as part of the national effort to build local capacity, strengthen the institutions of research for development, and advance science-based development to impart livelihood change for smallholder sorghum farmers of Ethiopia.

5 - ACCOMPLISHMENTS:

A. Achievements by project activities:

Activity 1.1. Establishing a core-set of Ethiopian sorghum landraces. In cooperation with the Ethiopian Institute of Biodiversity Conservation, a collection of 1425 sorghum landraces were assembled representing the different sorghum growing regions of the country and different agro-climatic zones. Many were purposefully selected based on the input at the inception meeting and in follow up conversations with Ethiopian collaborators for contrasting characters with respect to *Striga* resistance and drought tolerance. These accessions were generally collected from notoriously dry or *Striga* plagued regions of the country. These were grown out early in the project for basic plant character evaluation (see below) and DNA extraction at the National Agricultural

Biotechnology Research Center at Holeta for whole genome sequencing in the United States. Understandably, but practically challenging, seeds of the accessions are restricted to sowing in Ethiopia, so all whole plant evaluations had to be conducted there. The sequencing led to 879,407 potential SNP markers in the population for association studies involving drought and *Striga* specific phenotypes for this project and nutritional qualities (Tesso) and disease resistance (Mengiste) in collaborating projects sharing this core-set.

Activity 1.2. Phenotypic evaluation of core sorghum germplasm for drought and *Striga*. The collection was characterized in its first grow-out for basic descriptors in plant characteristics, including plant height, presence or absence of awns, glume cover at maturity, pericarp color, panicle exsertion, panicle compactness and shape, and also assessed male sterility reaction through fertility restoration tests. The latter was done by test crossing an unknown landrace genotypes onto a known male sterile line based on A1 cytoplasm (ATx623) to determine whether the landrace genotypes were sterility maintainers (B) or restorers (R) of fertility. This base-line descriptor characterization data provided a good supplement to the scant passport data in the existing record (there was little for most accessions aside from where they were originally collected) and began the sorting process for the germplasm line's desirability. This was a good accomplishment. Our intention was to also screen these for drought reaction but this required multi-location testing and the large group of accessions but with the preoccupation of the team with collecting an exhaustive descriptor which was partially fulfilled (causing tough competition for human and financial resources in the EIAR program) made this difficult. For the general plant characteristic observations and DNA extraction, the plants were grown in well managed favorable conditions in order to measure and sample from healthy unstressed plants. The Ethiopian collaborators were occupied with other matters and so were unable to grow and monitor the larger collection in drought stress environments. A subset was included as checks during improved hybrid and variety trials, but phenotypic data reflecting drought tolerance for a GWAS was not generated for the complete core-set of sequenced accessions.

Even though we had to defer the desired drought characterization data future dates (now), we were able to screen the entire 1425 Ethiopian sorghum landrace accessions for *Striga* resistance with a PCR-based marker that detects all known *lgs1* alleles described in our most recent publication by Gobena et al., 2017 PNAS 114:4471-4476. Mutations at *LGS1* alter the strigolactones exuded from the roots of these mutants such that most *Striga* do not germinate in their presence. The mutations described are a bit unusual, in that they mostly represent huge deletions (up to 34kbp). As such, they are a bit challenging to detect through high-throughput genotyping methods like GBS which are designed for detecting SNPs and small indels. Some 2000 leaf samples (1 from each accession) were taken from a grow-out of all accessions at Arsi Negele. This included the sequenced 1425 plus some wild sorghum accessions, a few checks and released Ethiopian regional and national varieties at various stages of improvement. The set was targeted for testing of plant disease reaction. The marker screen targeting *LGS1* was conducted at our lab at Purdue. About 6% showed mutation at this locus, indicating that the trait of low *Striga* germination stimulant activity based on *lgs1* exists in some native landraces.

Additionally, sorghum landraces from Ethiopia were screened for low *Striga* germination stimulant activity as possible source material for resistance breeding. This was accomplished on a subset of the Ethiopian core-set, specifically chosen from *Striga* endemic areas. These 215 landrace accessions were screened by a master's student, Tocuma Guta from Haramaya University, at the Holeta *Striga* screening facility that we established. Tocuma screened these with both the agar gel assay (phenotype) and molecular marker (genotype) specific for *LGS1*. He also found variability within most accessions. Nearly one third of the landraces he screened contained individuals which by the agar gel assay were classified as having low *Striga* germination stimulant activity. A subset of these, about a third, showed *lgs1* alleles by the marker screen. Again, this is likely partly due to the heterogeneous nature of the individual accessions and may also indicate new mutant alleles at *LGS1* and other mutations affecting SL biosynthesis and exudation that reduce the *Striga* germination stimulant activity. Perhaps more importantly, we have trained target country scientists in these efforts and demonstrated the potential value of native varieties as sources of *Striga* resistance that they may exploit through targeted breeding efforts informed by laboratory methods. There was overlap and agreement for 86 of the subset screened by Tocuma with respect to the *LGS1* marker screen on the larger set.

Activity 2.1. Introducing marker assisted selection to the breeding effort in the Ethiopian sorghum program. The PCR-based marker targeting *lgs1* mutations described above was used to train Ethiopian sorghum breeders and young scientists to provide some experience in marker assisted selection. Because the difference between *lgs1* (a mutation resulting in the phenotype of low *Striga* germination stimulant activity) and *LGS1* (high *Striga* germination stimulant activity) alleles are distinguished by a PCR product size polymorphism, the entire process from DNA extraction, PCR and detection of amplicon sizes by gel electrophoresis and UV imaging, could be done at the National Agricultural Biotechnology Research Center at Holeta. During two hands-on training sessions there, we demonstrated use of this marker on a segregating population of sorghum differing for *Striga* germination stimulant activity to show that one could detect *Striga* resistant individuals, at least with respect to that particular resistant trait, without field screening. Although multiplexed SNP markers might be more practical in the long-term, particularly when selecting for multiple traits in combination, those methods require sequencing which must currently be outsourced. We felt that this experience of following the entire marker screen in-house (at their Holeta facility) on native material in their actual sorghum improvement program for which some of them knew the difficult, heavily environmentally influenced field screening for *Striga* resistance, would appreciate the precise selection capability offered by such a technology. That the experience did hit home for some was evident from requests by two plant breeders, one professor and two graduate students in Ethiopia for primers specific to this marker and the instruction manual presented to the participants of the Holeta trainings.

Activity 3.1. Introduction and evaluation of experimental sorghum hybrids and open pollinated varieties of sorghum from multiple sources. Our project targeted sorghum germplasm development and use through two approaches, conversions from Ethiopian landraces as well as introduction of improved germplasm from a variety of sources. As the unavoidable lag in line development from core-set landraces proceeded, we initiated the process for the synthesis and development of sorghum hybrids using a wealth of improved sorghum germplasm at Purdue from decades of USAID investments through the INTSORMIL program. Through this process, the experience of developing and evaluation of elite sorghum hybrids was shared with the national and regional sorghum improvement programs through sets of introduced hybrid germplasm from our breeding program. These "1st generation" of sorghum hybrids came from our years of drought, yield and adaptability testing at Purdue and in our off-site nurseries. The *Striga* resistant hybrids were introduced through our Bill and Melinda Gates Foundation project. To anticipate the varying needs of farmers across the differing agro-ecologies of the country, particularly with respect to drought and yield, we introduced several categories of elite hybrids for evaluation. Hybrid sorghum introductions through SMIL began in 2015 for observation and comparisons with local checks in preliminary yield trials. Several categories of sorghum hybrids were introduced. These included a group of sorghum hybrids we dubbed, red and white hybrid groups based on kernel phenotypes, early season drought tolerant hybrids, Stay green late season drought tolerant lines, as well as dual purpose sorghum hybrids (suited for high yield as well as for and some forage hybrids including sorghum × sorghum hybrids and sorghum × Sudangrass hybrids (both with brown midrib and/or sweet stalks) introduced for testing at the request of EIAR and agricultural ministries to address national agenda. Each of these groups of hybrids were grown at several sites each season, including Shoa Robit, Shiraro, Miesso, Errer, and Kobbo). Several of these entries have since filtered through the Ethiopian national sorghum improvement program's stepwise testing procedures, leading to the official release of one outstanding sorghum hybrid (red kernel) as ESH 4 in 2016, and another one (white sorghum kernel type) released during this current year (2018).

Activity 3.2. Introduction and testing of parental lines (seed parents and pollinator lines) of sorghum for adaptation across target ecologies through organized field testing and data collection. In a similar way, elite parental lines with predicted good adaptation and good combining abilities were introduced to the Ethiopian sorghum program for evaluation and test crossing with their pipeline and finished varieties while the long-term process of new line development from the core-set played out. The parental lines chosen for introduction were known to produce superior hybrids in the United States and also in tropical test locations used through the years in our breeding program. These were generally chosen based on superior grain quality and drought tolerance both within the lines themselves and in hybrid combination. The A/B pairs on the seed parent side were also excellent in apparent yield potential exhibited in their panicle numbers and panicle size, days to maturity and height stature to practical hybrid production. This was deliberately done in part to counter what is described under Activity 3.3 as seed parent lines were advanced blindly from core-set landraces without regard to those qualities. Beginning early in the project in the 2013 season, the following introductions were made: **B-line seed parent** adaptation test (173 entries planted at two locations) and **R-line pollen parent** adaptation test (289+25 local entries planted at two locations).

Activity 3.3. Initiate parental line development for the hybrid program from local Ethiopian sorghum germplasm. In the initial grow-out of the core-set, any landrace that nicked with the plantings of the A1 cytoplasm tester ATx623 were test crossed to determine whether they were maintainers (B) or restorers of fertility (R). Hybrids with ATx623 were grown to flowering the following season and checked for male sterility. Those accessions producing male sterile hybrids were put in a B-line development pool for eventual seed parent development. Those resulting in male fertile hybrids were put into an R-line development pool for pollen parent development. Of the core-set test crossed (1341 of the original 1425), 103 accessions yielded F1s that were uniformly male sterile (potential B-lines) and 457 accessions were uniformly male fertile and gave full seed set. The remainder were mixed, partially sterile or gave poor seed set and were therefore not advanced. For the B-line development pool, sterilization continued by backcrossing onto A-line cytoplasm ([ATx623 × the original accession] F1), but initially without due regard to line improvement. This resulted in A/B pairs that were more homozygous than individuals in the original accession, but the pairs tended to be too tall and late for useful hybrid seed parents. Attention to recombining the B-lines under development among themselves or more often with elite B-lines introduced from Purdue occurred later in the project but corrected the course of line development and salvaged the desirable traits represented among the original landraces. Similarly, improvements to the pollen parent pool was effected by crosses with shorter, "tamer" usually introduced R-line germplasm early in line development before selfing to homozygosity. As potential B-lines were advanced and sterilized and potential R-lines emerged, these were tested for heterosis among each other and (more often) with introduced and proven A-lines and R-lines with good adaptation and combining ability. From this emerged and continues to emerge some accessions that make good hybrids. Unfortunately, it is too soon to tell the potential contribution of all landraces captured in the core-set to hybrids suited to particular agro-ecologies in the country. But the process is now the experience of sorghum breeders in Ethiopia.

Activity 3.4. Established a coordinated stepwise synthesis and testing of new hybrids developed from introduced and local sorghum germplasm to evaluate and deploy hybrids for target ecologies. The overall scheme for testing introduced material was to pull together a large set of experimental sorghum hybrids synthesized both at location in Ethiopia or introduced from Purdue and subjected in a series of testing and evaluation filtering the best products to come through the multiple evaluations, all along collecting data over seasons on these hybrids. The base material for the development of these hybrids were improved germplasm introductions from Purdue University. From this material, collaborating scientist selected promising looking material advancing them through a series of nurseries, as observation nursery, preliminary yield trial, advanced yield trial, and eventually that filtered through at this stage were put into a national yield trial that is evaluated in the wider range of environment in the country.

Collaborative field testing and validation of *Striga* resistant and drought tolerant sorghum lines and hybrids was conducted with Ethiopian national and regional agricultural research entities. In coordination with our Ethiopian collaborators, we established sites for targeted field-testing with agronomists and breeders for promising products emerging from their own breeding programs. Varietal evaluation procedures at EIAR and Regional BoA programs appear more accelerated than in previous years. In the past, programs often conducted testing over seasons and regions more rigorously in a stepwise testing program. The variety testing has been expedited by trials at multiple locations in the same year. Our *Striga* testing was conducted similarly. Although we did not develop *Striga* hotspots through artificial inoculation as originally planned, we used multiple locations in Humera, Shiraro, Abergele, Kobbo, and Feddis. Infestation appeared heavier and more uniformly distributed at the Humera location than at the other test locations. Drought stress was present at most of these locations also.

Activity 4.1. Devising crop management practices that enhance synergy with crop cultivars for maximal yield. Legumes were identified that potentially would cause suicidal germination of *Striga asiatica* and *S. hermonthica* suitable for intercropping with sorghum in target regions. Losses to *Striga* are most severe in nitrogen poor soils, typical of unfertilized plots of the poor. Control measures against *Striga* are limited and usually not accessible to subsistence farmers either because they are too costly or do not fit well with traditional agricultural practices. Intercropping with certain non-food legumes offers some suppression of the weed in cereal crops because of fixed nitrogen and allelopathic effects of the non-host legume. However, the practice is not widely accepted because the intercropped legume is either hard to establish or itself becomes a weed. To find a more suitable alternative, we collected root exudates from 52 Tanzanian common bean (*Phaseolus vulgaris*) accessions held by the U.S. National Plant Germplasm System and tested these for their effect on *S. hermonthica* under controlled laboratory conditions. This investigation was done mostly by a student helper in our lab, Eli Hugghis, a member of Minorities in Agriculture, Natural Resources and Related Sciences. The exudates were extracted from paper rolls in which the seedling beans were grown in the greenhouse. These were concentrated and then applied to conditioned *Striga* seeds embedded in agar either alone or concurrently with an artificial *Striga* germination stimulant. Exudates from the majority of bean accessions screened had little or no effect on *Striga* when compared to sorghum. Three accessions, however, were able to stimulate *Striga* germination, which, because beans are not a host, could offer control by causing suicidal germination of *Striga* seed if planted before the cereal. Two other accessions showed inhibition on *Striga* germination and early development. These bean varieties could offer a more acceptable protection from *Striga* as cereal intercrops than weedy non-food legumes. *Striga* suppression would be accompanied by the benefits of improved soil nitrogen content and additional high protein food in fields of subsistence farmers.

Food legume intercropping was evaluated for the control of *Striga hermonthica.* Legumes have received attention as companion plants that increase nutrient supply in the soil, improve soil physical characteristics, conserve soil moisture, deplete the *Striga* seed bank and reduce *Striga* emergence. A field experiment was conducted by a MSc student at Bahr Dar University to identify the most effective intercrop species/varieties compatible with sorghum, and determine the effect of intercropping system on *S. hermonthica* in eastern Amhara. Treatments consisted of soybean, haricot bean, cowpea and groundnut with three varieties of each crop accompanied with a satellite treatment of only sorghum for comparison. Treatments were evaluated under naturally *Striga* infested (hot-spot) areas of Kobo and Cheffa. Growth and yield parameters, economic analysis and land productivity were investigated. At Kobo, sorghum leaf area, plant height and leaf area index were significantly influenced by intercropped legumes. At Cheffa only plant height and panicle length were significant. The sorghum-haricot bean (cv-Lehode) intercropping recorded the highest grain yield (3177kg/ha), thousand-seed weight (41g), grain yield per plant (78.3g) and head-weight per plant (105.4g) at Kobo. On the other hand, at Cheffa the highest grain yield (3150kg/ha) and grain yield per plant (73.2g) were recorded from haricot bean cv-Nasir, while the highest head-weight per plant (105.4g) and thousand-seed weight (41g) from haricot bean cv-Lehode. *Striga* populations were influenced by legume varieties planted. At Chefa, cowpea variety Tentekit intercropped with sorghum had the least number of emerged *Striga* (18 plants/plot) without significant difference with Bekur, Bole and Lehode, and the highest number of *Striga* population (51 plants/plot) was recorded from soybean variety Wollo. At Kobo, the association of sorghum with cowpea variety Bekur, Tentekit and bean cv-Lehode had minimum *Striga* population (31, 34 and 39 plants/plot, respectively) and groundnut variety Eta had the most *Striga* plants (135 plants/plot). The partial land equivalent ratio (PLER) and gross monetary value (GMV) analysis indicated significant differences due to the effects of intercropping sorghum with different legume varieties. At Kobo, the highest partial LER (1.34) of sorghum and GMV (19508.6 ETB ha-1) was obtained when bean variety Lehode intercropped with sorghum and the soybean varieties and cowpea cv-Bole showed poor compatibility for intercropping with sorghum. However, at Cheffa, bean variety Nasir gave the highest partial LER (1.4), and the highest GMV of 21688.4 ETB ha-1 was recorded when groundnut variety Sedi was intercropped with sorghum without statistical difference with Fenta (21108.0 ETB ha-1) and bean variety Nasir (20328.2 ETB ha-1). In summary, intercropping sorghum with haricot bean (cv-Lehode and Nasir) and cowpea (cv-Bekur and Tentekit) could be strategically combined with other approaches for integrated management of *Striga* to stabilize and improve sorghum production in the subsistent agricultural system of eastern Amhara.

The influence of cowpea and soybean intercropping pattern in sorghum on *Striga* infestation and system productivity at Mechara, eastern Ethiopia was tested. A field experiment was conducted at Mechara Agricultural Research Center by a MSc student from Haramaya University to determine the effect of cowpea and soybean intercropping on *S. hermonthica* infestation in sorghum and to assess the effect of *Striga* and intercropping on system productivity. Two legume crops (soybean and cowpea) planted simultaneously with sorghum and at first weeding of sorghum and three planting pattern double alternate plants; two rows in between two rows of sorghum and both double alternate plants and two rows in between two rows of sorghum. Cowpea and soybean reduced the *Striga* infestation level. Cowpea (84.9) was more effective in reducing *Striga* infestation than soybean (65). Analysis of variance revealed that delayed planting of legumes until the first weeding in sorghum increased days to heading and maturity of sorghum by 4.7% and 2.7% respectively. Intercropping sorghum with cowpea and soybean did not significantly influence days to heading and maturity of sorghum. Both time of planting and planting pattern did not significantly influence sorghum plant height, while legume crops significantly influenced sorghum plant height. Both cropping systems significantly influenced sorghum grain yield when compared to sole sorghum cropping systems (2348.6 kg ha-1). Sorghum/soybean cropping system reduced sorghum grain yield by 23.9% whereas sorghum/cowpea reduced by 40.31%. The highest grain yield was obtained from the combination of sorghum intercropped with soybean at the first weeding of sorghum and the lowest grain yield was obtained with cowpea intercrop planting simultaneously. The highest soybean grain yield (1169 kg ha-1) was obtained when soybean was planted simultaneously with sorghum. The highest cowpea grain yield (1979.5kg ha-1) was obtained when sowing cowpea simultaneously with sorghum in 2R planting pattern and the lowest was observed when sowing cowpea (1290.5kg ha-1) at first weeding or hoeing of sorghum. The total land equivalent ratio (LER) indicates about 55% relative yield advantages when sorghum was intercropped with soybean and 18.9% when intercropped with cowpea.

B. Major challenges encountered and resulting project adjustments

Our experience working with Ethiopian institutions and the support we have been receiving from the staff as well as their leadership has been excellent. There have been few minor challenges. One major challenge has been the attrition at leadership positions. Several leaders have moved for promotion, further education, or change jobs including assignment as minister of agriculture. All good reasons but significantly affected momentum. The pooled resources kept at SMIL to support in-country work by collaborators has not supported collaborators based on work load, perhaps an adjustment there may be useful. We each received additional requests that we had to provide for supplies that they needed. Another issue that had arisen was how well the efforts of all external funded projects were coordinated to create true synergy for our local hosts. While the locals have made it work, it may need some attention moving forward. The one event that affected our work or everyone's for that matter, is the weather where rainfall patterns have affected parts of our work significantly; but no simple solution can be devised for it.

More recently, the uncertainty in security situation had stopped us from traveling to project sites and even to the country (as in the 2017 crop season). On the balance, working with the Ethiopian government programs on sorghum research for development has been rewarding and positive.

C. Student training achievements

This project has supported training at Purdue University for one post-doctoral research associate originally from Nigeria, Adedayo Adeyanju and three doctoral students, Patrick Ongom from Uganda (partially funded) , Xiaochen Xu, from China and Habte Nida from Ethiopia (partially funded in collaboration with Tesfaye

Mengiste).

D. Short term training and outreach

Our capacity building effort in Ethiopia has gone well, and duly recognized. Among the most appreciated, and practically very useful exercise we have undertaken is annually is a "travel workshop" where each fall before crop harvest, we visit research plots and seed production fields together as a team of federal and regional staff from respective programs along with SMIL scientists interacting directly in the field exchanging views assessing situations in the field both positive and unfortunate developments that take place as a result of management practices, weather, or other unexpected events. On these same trips we get a chance to interact with farmers and learn about their experiences as well. On a more traditional training program we undertook in Ethiopia, the establishment of the *Striga* bioassay lab at Holeta National Biotechnology Research Center was a major capacity building effort in support of the *Striga* research in Ethiopia. This was supported mainly with funds provided by the Bill and Melinda Gates Foundation but benefitted graduate students and staff from the SMIL project. Field based germplasm screens are particularly challenging for *Striga* due to significant variation in year to year infestation levels and inability to accurately evaluate phenotypes before significant damage is done. In this project, we have established a *Striga* lab that is being used by local staff for screening sorghum germplasm. This facility is meant to expedite the screening of sorghum germplasm for resistance. One junior scientist was hired by the EIAR through the Ethiopian Government funds to support manage the *Striga* lab and supervise day to day activities of the lab. An assembly of laboratory supplies and equipment specific to *Striga* resistance laboratory screening protocols were procured at Purdue and shipped to Ethiopia to set up a *Striga* resistance screening facility at Holeta. Staff scientists from Purdue travelled to Ethiopia and conducted training programs for local staff. The first session in November 2014 was run by Dr. Patrick Rich on laboratory methods for screening germplasm for their resistance to *Striga*. The laboratory screening is used to identify variants of sorghum or other crop germplasm with reduced production of the chemical signal required for the germination of *Striga* seeds. Beyond the current project, the facility will be an asset for the national program in furthering indirect lab-based selection for *Striga* resistant varieties to complement observational data collected from field-based breeding work for sorghum and other crops that are parasitized by *Striga*. The second round of training was held in April 2016 and covered both the traditional germination assays and the high throughput methods for screening germplasm that was developed at Purdue. This later approach utilizes exudates collected from plants to assay germination inhibition. Over 20 graduate students and junior researchers and laboratory technicians from different universities, federal and regional research centers were trained. Female scientists made up 5-10% of the participants. Purdue scientists, Dr. Patrick Rich and Dr. Daniel Gobena developed the training module for the bioassays as well as the molecular markers training. A result, students and other EIAR staff are currently able to screen germplasm either from a breeding population or landraces for natural low germination stimulant lines.

Informal training was provided to EIAR, regional agricultural research staff and local sorghum producers during annual trips to research trial locations by the PI. These offered exposure of interested parties to improved germplasm and gave them the opportunity to discuss questions or problems with the project PI. This also included more formal directed presentations given by the PI and his associates. In addition to those mentioned above at Holeta, we conducted a one-day training on Hybrid Sorghum Seed Production to interested technical staff from both public and private organizations this past August. Topics covered included: the advantages of hybrids, use of male sterility systems in hybrid sorghum seed production, hybrid sorghum seed production process and seed systems as vital national institutions.

6 - UTILIZATION OF RESEARCH OUTPUTS

A. Technologies and management practices by phase of development

Ethiopia is a good place to lead a coordinated and disciplined agricultural research program efforts. Things do get done. We have introduced and advanced research technologies of improved *Striga* resistant sorghum varieties, drought tolerant hybrids, and more recently high yield potential dual-purpose sorghum hybrids as well as forage sorghum hybrids. A drought tolerant white grain sorghum hybrids was released earlier this year. The rest are at different stages in the step-wise testing program adopted by EIAR. Furthermore lines derived from the core-set landraces, are mostly still in the developmental phases as this is a long-term objective. Even through staff attrition for further education abroad, they sustain good efforts. The experience and training of Ethiopian national and regional agricultural scientists and of their university staff and students has through this project become one of ownership. The Ethiopian sorghum hybrid is getting stronger in the staff it is building and the facilities are improving as well. They have a good field staff with energy, are collaborative, and able to carry a large program and work in a more disciplined way. Even since we resurrected the hybrid sorghum work, they've shown that they could run a balanced program with eagerness to learn and adjust leading to releases of both OPVs and hybrids in a coordinated way. With return of trained personnel from Australia (Taye and Alemu) and Habte from Purdue next year, they will be more. They have shown that they work well with regional programs extension and seed enterprises. Having witnessed the gains achieved through exploitation of heterosis in hybrid sorghum, they appear energized and eager to take leadership in those places most familiar to them. We believe both farmers and those that serve them through the agricultural ministries have embraced our collaborative efforts to strengthen work in their native crop as a means to catalyze economic growth in rural communities.

B. Intermediate outputs

In much of the research we undertake, there are numerous outputs that we come up with that provide nuggets of information that we build upon in future research, they get in our many presentations that we make overtime and used as preliminary evidences. And in plant breeding programs, we often end up with genetic resources that are of certain reasonably good quality but not of quality that we may officially release for direct use by farmers or companies. They may be combined with other genetic resources that eventually would end up in a final product of commercial value. In any case, such outcomes are numerous to enumerate in most of our programs.

Educational materials about integrated *Striga* management (using soil fertility enhancement and water conservation practices along with improved drought tolerant and *Striga* resistant varieties) were produced in Ethiopian local languages during the course of the project including: *Striga* Management Manual by Melkassa Agricultural Research Center, EIAR; *Striga* Management Manual (Amharic language), SNNP; Leaflet on *Striga* Management (Afan Oromo), Oromiya Bureau of Agriculture; Leaflet on *Striga* Management by Sirinka Agricultural Research Center; Leaflet on *Striga* Management (Tigray language), Tigray.

C. Publications

Conference presentations:

Ejeta, G. (October 2014). Research for Development (R4D): Mantra or practice? Presentation at EIAR Crop Conference, Addis Ababa, Ethiopia.

Ejeta, G. (April 2018). Advancing science, technology, and innovation in sorghum, millets, and other traditional crops through transdisciplinary approaches to transform African agriculture. Sorghum in the 21st Century, Cape Town, South Africa.

Ejeta, G. (April 2018). Sorghum: Significance for food and nutrition security from a global perspective. South Africa National Research Foundation "Science for Society" Lecture. April 10, 2018, Cape Town, South Africa.

Ejeta, G. (April 2018). Enhancing resilience in the face of climate change. Sorghum in the 21st Century, Cape Town, South Africa, published.

Bayable, D., Adeyanju, A., Fuyou, F., D., Ejeta, G., & Mengiste, T. (April 2018). Broad spectrum and complete fungal resistance in sorghum conferred by an intracellular immune receptor. Sorghum in 21 Century, Cape Town, South Africa.

Guta, T., Rich, P., Mekbib, F., & Ejeta, G. (January 2018). Evaluation of Ethiopian sorghum landraces and wild relatives for pre-attachment resistance mechanisms to *Striga* infestation. Sorghum in the 21st Century, Cape Town, South Africa.

Fenton, M. & Ejeta, G. (January 2018). Genome-wide association analysis for *Striga* resistance in sorghum MAGIC population. Sorghum in the 21st Century, Cape Town, South Africa.

Mengiste, T. & Ejeta, G. (April 2018). Identification and mapping of anthracnose resistance genes in sorghum [*Sorghum bicolor* (L.) Moench]. Sorghum in the 21st Century, Cape Town, South Africa.

Nida, H., Mengiste, T., & Ejeta, G. (April 2018). Identification of sorghum grain mold resistance loci through GWAS. Sorghum in the 21st Century, Cape Town, South Africa.

Tessema, G. G., Nida, H., Tesso, T., Ejeta, G., & Mengiste, T. (April 2018). Sorghum genetic diversity study and gene mining for important traits. Sorghum in the 21st Century, Cape Town, South Africa.

Journal articles:

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7 - STATEMENT ON PROPOSED FUTURE ACTIVITIES FOLLOWING PHASE I RESEARCH ACCOMPLISHMENTS

A. Statement on future activities in Phase II

Phase II Activity 1. Evaluation of Ethiopian sorghum landraces for drought tolerance to produce phenotypic data for the trait to associate with the sequence data generated in Phase I: Genome-wide association of data on Ethiopian sorghum landraces with phenotypic measure for drought tolerance will be of great value to identify genes and gene function responsible for drought tolerance in Ethiopian sorghums. Given the large set of Ethiopian sorghum landraces have been sequenced in phase I, the next stage of project activity will be to phenotype the same germplasm collection entries for a number of vital adaptive traits including drought tolerance resistance. The task of evaluating the core-set of landraces assembled and sequenced in Phase I (1425 accessions) for drought reaction / tolerance will be conducted in groups of landrace germplasm sampled as 200-400 entries. The activity will be organized as graduate student projects involving applicants from national programs and studying at Ethiopian universities. For the drought project we will recruit three graduate students each taking one set of the 200-400 entries to be evaluated under 2-3 rainfed environments over two crop seasons. In addition to the selected sets, the genetic resources will include both local and national check cultivars known for their drought reactions. Standard agronomic practices and experimental designs will be used. In addition to standard agronomic traits of height, maturity, and agronomic aspect scores, data on drought related physiological measures and in those locations specific to the student will be gathered. Yield under stress will be the primary phenotypic data to be used in the eventual GWAS. Data from the three students (600-1200 entries) will be pooled for eventual characterization and analysis. Pooled phenotypic data will be layered upon the GBS data acquired in Phase I on the core-set in coordination with a post-doctoral research associate who will direct the bioinformatics. The individual trials will be multi-environment all in the same crop season, although invariably there may be a failed trial at one or more location, and the trial may have to be conducted over 2-3 crop seasons. Wherever possible, we will look into the possibility of growing the trial under rainfed and irrigated condition during the same crop season to conduct a comparative analysis as indicator of drought effect. Genome wide association study (GWAS) for drought tolerance will be conducted. The data will be processed by the post-doctoral scientist in the project both in subsets particular to each student's project and pooled over the entire core-set. It is hoped that genes controlling drought tolerance will be discovered through these activities and that molecular markers will ultimately be developed from this gene discovery for more efficient selection of drought tolerant sorghum through an expedited breeding process.

Phase II Activity 2. Developing parental lines for hybrid program from Ethiopian Sorghum Landraces: We have tested the large collection of Ethiopian sorghum landraces for fertility reaction by a series of test crosses with standard A1 cytoplasm male sterile line Tx623. A total of 103 landraces were identified as good B lines that can be developed to be seed parents, and 457 landraces that can be developed into R lines (pollinator parents. Each will be intercrossed with sets of selected improved lines, to convert the Ethiopian landraces into shorter and earlier versions that still has the Ethiopian landrace base of genomic proportion for desired traits. Native parental lines will be advanced through progeny generated within both the B-line and R-line development pools involving intercrosses with Ethiopian landraces derived from accessions, either directly descended from or intercrosses among the core-set from Phase I. Crosses of these with elite well adapted introductions will also be advanced toward development of seed and pollen parental lines with good combining ability targeted for (and adapted to) drought-prone agro-ecologies within the country. Particular attention will be given to improved yield and quality traits demanded by local farmers along with yield stability under water stress. Practical issues of maturity, plant height, cleanly expressed male sterility and head size in the seed parents will be given due attention toward efficient hybrid production. Those in the B line background will be converted to male sterile lines, and the R lines will be used as pollinators. The resultant progenies will eventually be parental lines for a new generation of hybrids made up of materials with large proportion of genomic contribution.

Phase II Activity 3. Stepwise synthesis and testing of experimental hybrids from Ethiopian Sorghum Landraces Improved for multiple agronomic traits: For full exploitation of the power of sorghum hybrids to impact sorghum farming in Ethiopia, the national program will need an infusion of elite parental lines to their program. The most significant contribution that SMIL has made is to share with the EIAR a wealth of improved sorghum germplasm of elite parental lines with drought tolerance, broad adaptation, and desirable grain quality that was made possible through decades of USAID investments in the INTSORMIL program. SMIL activities through this project have leveraged that investment to revitalize the Ethiopian sorghum improvement program towards embarking on a commercial sorghum hybrid program. In addition to the supply of such valuable germplasm, we provide on the job training and capacity building in breeding sorghum hybrids as well as in the art and science of hybrid seed production (next section). While longer term, the EIAR sorghum breeding program would begin production of experimental hybrids based on local sorghum germplasm, in the immediate future the program can catalyze increased sorghum grain supply through an accelerated sorghum hybrid development and use based on quality germplasm from introduced sources. Hence, our project can support EIAR on both fronts. First the use of local landraces in the development of parental lines for hybrids based on improved A & B lines (seed parents) and improved R-lines (pollinator lines) derived from the core-set of Ethiopian landraces would be undertaken. Such lines will be intercrossed in hybrid combinations to determine parents that have superior combining ability. Individual hybrids will be evaluated for yield and adaptation and evaluated in a stepwise testing procedure to determine winning combinations. Secondly, the project will assist through joint efforts using breeding facilities at Purdue and at EIAR, the continued development and evaluation of experimental sorghum hybrids using SMIL germplasm from Purdue and other sources. Experimental sorghum hybrids will also be jointly evaluated at different environments in collaboration with regional bureaus of agriculture in Ethiopia to select ecology specific hybrids that fit in the different growing condition of the country. Different combinations of hybrids will be characterized for yield and tolerance to drought as part of the national testing scheme, but also evaluated for food quality and other value chain development pathway that may be available. Hybrids could be developed for specific target environments, or an assessment could be made to choose the best parents that may produce good hybrids for most environments. It was such an approach that led to releases of first generation sorghum hybrids that are currently ready to be piloted. There is current interest for using sorghum for beer, and potential animal feed processing may be the next target. Substantial yield gains through heterosis are expected. Stepwise testing following the model set forth and experience gained in Phase I will continue toward ultimate release of sorghum hybrids coming through the breeding pipeline through the Ethiopian national and regional agricultural research organizations.

Phase II Activity 4. Promote and support the development of a hybrid seed production system in Ethiopia: Availability of an outstanding cultivar is often a vehicle for change in agricultural, as it serves as catalyst for use of other inputs and associated improved farming practices that would arise towards boosting productivity. With recent releases of superior hybrids that combine drought tolerance with higher yields and desirable plant architecture, we propose to put together a collaborative team of scientists at EIAR and Regional Bureaus of Agriculture, in each of their respective divisions of research, extension, and seed production to layout scheduled testing, demonstration, popularization, and seed production in an interconnected and seamless sets of duties in a coordinated fashion. We have used such a system for OPVs before, and good precedent has been set. Parts of that experience in breeding, testing, and piloting could easily carryon. But the nature of sorghum hybrids, and their seed production system are distinctly different requiring a new series of collaborative engagement to make it stick. It will be a mistake to ask the local seed enterprises to take up hybrid seed production without technical support that SMIL scientists can provide to build experience on the ground leaving them with some confidence after a few years of getting that underway with much of the kinks taken care of well. The development of an efficient and profitable seed system in Ethiopia will be a must for the technology to impact livelihoods. Such project will need to be encouraged and supported as a vital outcome of a research and development initiative. Proper division of labor will be developed for each participating component programs at each of the institutions to replicate a system and approach that we had used with OPVs. EIAR will provide back up by taking on the responsibility of basic breeder seed production and supply, in addition to their regular synthesis and distribution of new experimental sorghum hybrids to keep the pipeline going. Research programs in each region will take on foundation seed production and supply to support their respective seed enterprises with fresh foundation seed supply each year. The extension section of each regional bureau will be in charge of hybrid demonstration, farmer training, and popularization towards generating interest in seed demand. The seed enterprises of each region, as well as private sector groups in the respective regions will be encouraged to produce 'certified hybrid sorghum seed' to sell and distribute competitively in each region and potentially across the country. Such assignments of technical and physical charges will be supported and accomplished through organizing annual training programs for sorghum hybrid seed production for public research, seed enterprises, and private seed growers. We will also be recruiting and training public and private hybrid sorghum seed producers at each region where such trainings occur. The performance of the planning and undertaking a scheduled seed production of both foundation and certified seeds of parental lines and hybrids with each producer organization will be monitored and evaluated. We will also be promoting good agronomic management in production and demonstration plots engaging agronomists at local bureaus and universities working together with the extension services of their respective regions. We will be working closely with existing regulatory agencies to schedule inspection and monitoring of hybrid sorghum seeds scheduled for distribution and sale. Proper training of seed producers is a must supplying them with seed handling procedures of parental and hybrid seeds beyond production to track identity and integrity of seed through proper seed conditioning protocols. In conjunction with these activities to promote quality hybrid sorghum seed production, we will encourage development of a sustainable seed system based on hybrid sorghum. Toward this goal, we will recruit private seed producers to engage in hybrid seed production and marketing, engage seed businesses in hands-on production and processing of sorghum hybrids seeds and arrange for seed business training for keen producers through hired consultants.

Seed production of ESH-4 above is currently underway in several locations in Ethiopia. Photo above is from ACRE Indiana.

B. Linkage to Phase I objectives and activities

Each of the above proposed activities are directly linked as follow up actions to advance progress made in the project objectives and activities in phase I. Phase II activity 1 advances the genomic sequencing data generated in Phase I by producing phenotypic data for a very desirable trait of drought tolerance to allow identification of genes and gene function for drought and drought related traits. This activity will continue the long-term goals set forth in Phase I to increase sorghum yields in quantity, quality and stability and improve the livelihoods of farmers growing this native crop. Phase II activity 2 follows from the evaluation and characterization of the Ethiopian sorghum landraces for potential use as parents (seed parents and pollen parents) in the planned sorghum hybrid development program. As mentioned above, the Ethiopian sorghum germplasm has been characterized for these groups in Phase I. Sorghum hybrids are routinely produced on a cytoplasmic-genetic-male sterility system. Activity 2 is designed to guide the development of proper parental lines (male sterile A lines as well as pollen producing male parent R lines to restore fertility in the eventual hybrid) for the production of sorghum hybrids from Ethiopian sorghum landraces. Phase II activity 3 follows from the synthesis and production of experimental hybrids that we advanced leading to the release of two sorghum hybrids with drought tolerance. Good research programs need to have good pipelines of research results to assist with overcoming potential problems that may arise. A release of a cultivar is not an end on itself. New sorghum hybrids with even more superior trait combinations need to be developed to replace past releases, as what our work had shown with the release of the current sorghum hybrids in the country. Phase II activity # 4 focused on establishing the impact pathway for the mechanism of hybrid seed production, use of seed of released hybrids for demonstration, popularization, piloting, and scale up of sorghum hybrids produced and released in Phase I for its eventual adoption and wide use. Each of these activities will be accomplished through coordinated joint participation and implementation with gained participatory knowledge of the genetics of drought adaptation among Ethiopian sorghums, improved testing, evaluation and release of introduced and native 1st and 2nd generation varieties and hybrids with improved crop management practices, as well as the development of a functional and viable seed industry in Ethiopia that will feature and promote the delivery of high quality sorghum hybrid seeds of superior quality delivered on time.

C. Training and outreach objectives

Each of the four activities described above are to be accomplished with solid elements of training and objectives at different levels. Training described above include degree training for you Ethiopian professionals at Ethiopian universities, mostly at the MSc level in connection with genome wide association studies for drought tolerance for the exploitation of Ethiopian sorghum landraces. Training also includes on the job capacity development of research scientists, extension professionals, as well as staff involved in the public and private seed enterprises introducing them to the science of the hybrid sorghum development and use. We will work with sorghum breeders to impart on them the discipline and care needed to synthesize, evaluate, and produce parental lines and basic seeds for the production of sorghum hybrids. We will work with scientists at both EIAR and regional bureaus. We will engage extension professionals as they work with farmers in introducing new hybrid seeds and share their experiences via field days to other farmers and government officials to generate buy-in for support of the hybrid seed program and agriculture. Training and outreach will be with the seed enterprises too as discussed in sections above. It is apparent, therefore, that the success of each of the Phase II activities are premised with deliberate and organized training and outreach, without which success could not be guaranteed. This is particularly so, as experience in hybrid sorghum is limited in Africa, and careful education and experience sharing will be required to ensure that the activity ends with good traction on the ground for sustainability. Embedded seed production and processing, quality control, seed conditioning, seed treatment, and general care in handling hybrid sorghum seed of great value to be provided at a good price. To sum up, the above activities all embrace some aspects of training and outreach, both for graduate students through their collaborative research to discover sorghum traits and genes controlling drought tolerance, for breeders to develop products, and for agronomists and extension agents to impart good management practices, and to the greater community of practice of private, public, and civil society to develop a more nuanced understanding of the value of a good seed. Also needed is a streamlined development and testing of superior parental lines as well as the production and sale of certified sorghum hybrid seed of desired quality to be delivered on time; and for seedsmen and entrepreneurial farmers to amass the experience and confidence to produce quality seed and generate demand for profitable seed distribution.

IMPROVED CROP GENETICS AND PROCESSING METHODS FOR INCREASED PRODUCTIVITY AND NUTRITION FOR SMALLHOLDER SORGHUM PRODUCERS IN ETHIOPIA

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Solomon Assefa (Sorghum breeding) - *ARARI Sirinka Research Center, Sirinka, Ethiopia*

3 – PROJECT GOALS AND OBJECTIVES

The goal of the project was to develop and deploy improved technology options (high yielding varieties/hybrids and processing methods) that will increase productivity and nutritional value to enhance food and nutritional security of smallholder sorghum producers in Ethiopia.

The objectives of the project are:

1. **Development and systematic evaluation of core sorghum germplasm population for nutritional quality traits**
    a. Assembling core germplasm population of Ethiopian sorghum
    b. Phenotypic evaluation of core sorghum germplasm for agronomic traits and nutritional attributes
2. **Develop and deploy high yielding locally adapted sorghum varieties/hybrids with enhanced nutritional value to promote food security, health and nutrition for smallholder farmers**
    a. Characterize sorghum germplasm for grain quality characteristics and nutritional value
    b. Characterize the protease inhibitor proteins and identify genetic variants with reduced activity of protease inhibitor(s)

c. Developing sorghum varieties/hybrids with superior yield potential, agronomic adaptation and enhanced nutritional value

3. **Optimize traditional sorghum processing methods for increased availability of proteins and calories in sorghum based diets**

    a. Evaluate the effect of variety and traditional food processing on PI activity, anti-nutrient levels, and digestibility of proteins and starch in sorghum

    b. Optimization of sorghum food processing techniques to enhance protein digestibility

    c. Promoting varietal technologies, and improved food processing methods for improved production and utilization

4 – OVERVIEW OF ACTIVITIES

Activities carried out under the three research objectives listed above produced expected outputs that serve as intermediate result for planning and implementing follow up research. The assembly and characterization (phenotypic and genotypic) of 2000+ accessions representing the sorghum diversity in Ethiopia were systematically assembled. The accessions were evaluated at multiple environments over three-year period where data on diverse agronomic characteristics as well as grain and nutritional quality parameters were assembled. These information is available for use by all interested groups. The determination of fertility reaction of the subset of the accessions and the genotypic data generated on 1600 of the samples is an important resource for use of these materials in genetic improvement of the crop for Ethiopia and the region.

The identification of protease inhibitor genes and the discovery of novel alpha kafirin alleles associated with protein digestibility are significant milestones in sorghum protein nutritional quality research. These results constitute among the major pieces of the big puzzle surrounding sorghum protein digestibility. Another important result is the presence of negative correlation between protein content and protein digestibility. Genotypes with high protein content and high gamma kafirin fraction tend to have lower percent digestibility than those with lower levels of protein and gamma kafirin.

Moreover, the food process optimization to enhance protein digestibility has generated encouraging results. All food processes reduced protein digestibility but the reduction was the least in roasted and fermented food products. A further optimization effort through increasing fermentation time and reducing the flour particle size markedly improved protein digestibility of fermented bread. Follow up activities are underway where additional grain pre-treatment processes such as sprouting, decortication, and roasting are combined with fermentation and flour particle size to determine their effect on protein digestibility. The complete data on this will be available before the end of the year.

5 – ACCOMPLISHMENTS

A. Achievements by project objectives

Objective 1: Development and systematic evaluation of core sorghum germplasm population for nutritional quality traits

Obj 1.1: Assembling core germplasm population of Ethiopian sorghum

The assembly of ~2500 Ethiopian sorghum germplasm population was completed in the first year of the project. This activity was performed in collaboration with Drs. Ejeta and Mengiste and the Ethiopian Biodiversity Institute. Activities in subsequent seasons focused on purification of the samples, removal of redundant materials and seed increase of the collections that the final number reduced to 2100 collections. The germplasm accessions represented all sorghum production regions of the country, altitudinal zones, agro-ecological spectrum and utilization attributes. These collections are expected to serve as core germplasm representing sorghum diversity in Ethiopia and is of crucial value for future sorghum improvement efforts in Ethiopia and the region. In order to facilitate the use of these materials in cultivar development, especially of hybrid breeding, about 1437 accessions from the collections were sorted into B- and R-germplasm pools through crossing with A1 cytoplasm female (ATx623). Of these, about 50% were found to be R-lines (fertility restorers) and 12% were B-lines (fertility maintainer). The remaining 38% were partial restorers.

Obj. 1.2: Phenotypic evaluation of core sorghum germplasm for agronomic traits and nutritional attributes

The purified collections (~2100) were evaluated at more than 12 environments over 3 three seasons. Data were collected on agronomic characteristics and grain quality parameters. As this is a joint objective between three breeding related projects, other parameters including drought tolerance and disease response were also collected. Overall, the collections displayed robust variability for various traits. In addition to the phenotype data, a good representation of the population (1600) were genotyped using genotyping by sequencing platform. Preliminary analysis of the genotype data grouped the accessions into eleven subgroups reinforcing the wide phenotypic variability observed from the field data. Genetic diversity and population structure analysis based on both DNA marker and the field phenotype data is completed and submitted for publication. In order to have a detail grasp on the population both genotype data and phenotype data were used to create sub-sample of the collection consisting about 500 accessions.

These sub-sample sets and other tropically adapted breeding lines were evaluated for various agronomic traits and several nutritional quality traits including starch, protein, fiber, as well as all mineral nutrients including Fe, Zn, Mn, Ca, Mb, and K. This activity identified a set of genotypes with 40-60% higher protein content and 20-40% higher protein digestibility than normal sorghums. Likewise, accessions containing high Iron and Zinc were also identified.

Objective 2: Develop and deploy high yielding locally adapted sorghum varieties/hybrids with enhanced nutritional value to promote food security and nutrition for smallholder farmers

Obj. 2.1. Characterize sorghum germplasm for grain quality characteristics and nutritional value

This is tied to activity 1.2 above. Yemane Belayneh, graduate student from KSU, was sent to Ethiopia and spent a semester (December 2016 to May 2017) to work on characterization of grain quality parameters and nutritional attributes using NIR methods. Due to restrictions on germlasm movement, all of the 2100 samples had to be ground in to a flour for shipment to the United States for nutritional analysis. As indicated in Obj. 1.2 above, a subset of the samples and additional breeding lines were subjected to various nutritional analysis. The data generated on these samples were used in MS thesis of Alemnesh Bekele as well on journal publication. The raw data has been reported to SMIL and is ready for uploading on public database.

Obj. 2.2. Characterize the protease inhibitor proteins and identify genetic variants with reduced activity of protease inhibitor(s)

Protease inhibitors were found to be among the culprits undermining protein digestibility in sorghum. Comparison of *in-vitro* protein digestibility score before and after protease inhibitor removal in raw sorghum flour samples resulted in a stunning difference in digestibility (Fig a). Efforts were made to purify, characterize and identify the genes coding for protease inhibitor proteins and use the information to screen sorghum for variants of protease inhibitor genes with reduced activity. Various treatment methods were used to understand the property of the PI compound. Size fractionation with HPLC indicated that the compound is a large molecule in the range 3.5 to 10 Kda (Fig b). Like most anti-nutritional factors, the PI compound appear to be concentrated in the bran layer of the grain such that different levels of decortication reduced the activity of the compound with the 30% decortication completely removing the PI (Fig c). The compound is shown to be highly stable under high temperature indicating its practical relevance to food applications. Boiling the samples for up to 60 minutes did not show any sign of reduction in PI activity which implies that the compound will continue to compromise protein digestibility in cooked products. Through various biochemical and enzymatic treatments, the compound was proven to be a protein that treatment of the compound with other protein digestive enzyme chymotrypsin completely eliminated the compound.

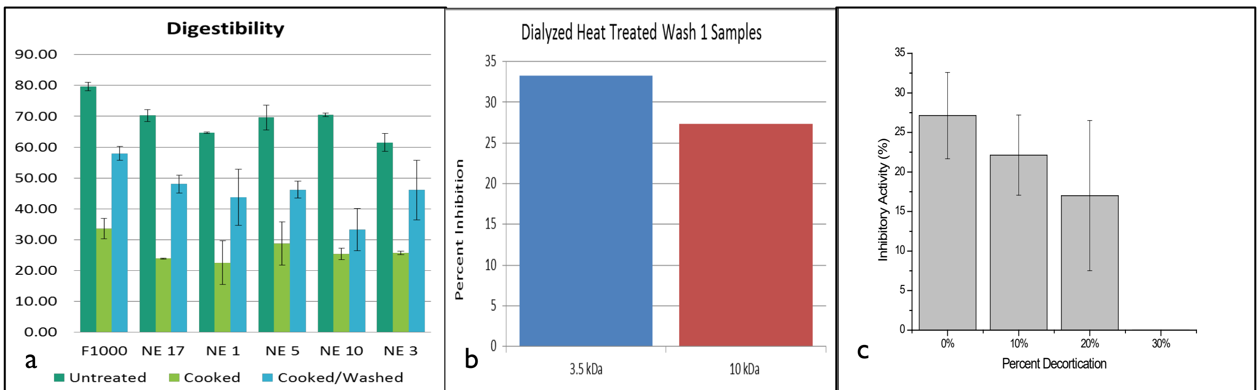
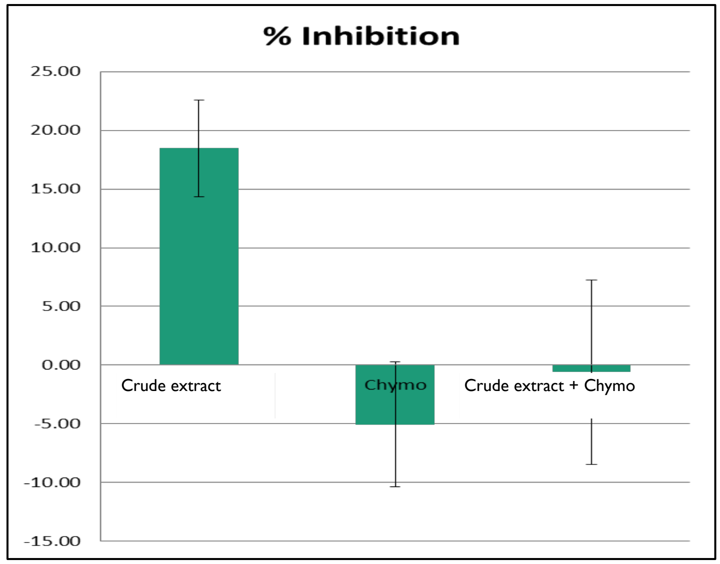


Fig. Characterization of protease inhibitor (PI) from tannin free sorghum genotypes; a) the impact of PI on protein digestibility; b) HPLC profile suggesting PI is a large molecule; and c) decortication test showing the accumulation of PI in the bran layer.



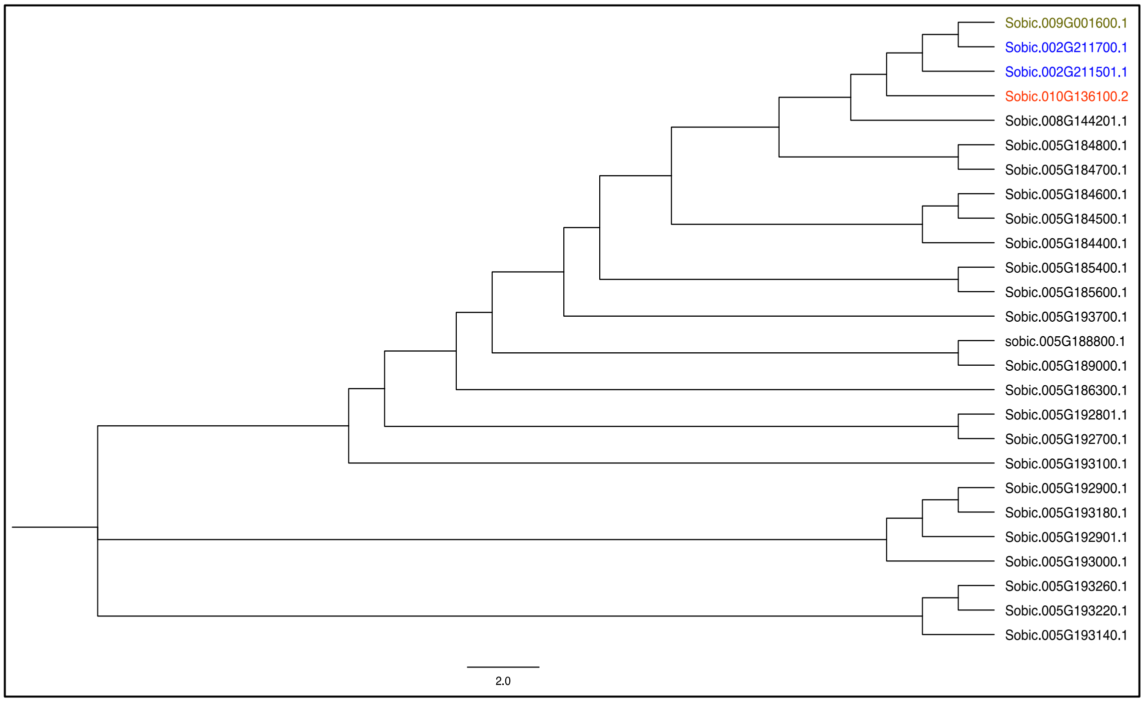
Following the confirmation through the action of chymotrypsin digestion (see figure below) that the PI compound was a protein, an effort was initiated to identify genes coding for the PI protein. After very lengthy laboratory process that took over two years, the PI compound was isolated from sorghum samples and purified for identification. The University of Florida Proteomics and Mass Spectroscopy service facility was contracted for protein identification. Six cysteine protease inhibitors were detected in the samples, four of them (Sobic.001G324500, Sobic.001G324700, Sobic.001G324800 and Sobic.001G487800) on chromosome 1 and two of them (Sobic.003G126800 and Sobic.003G400400) on chromosome 3 of sorghum genome. Primer sets targeting these genes were developed and the genes from 56 high and low digestible sorghum samples were amplified and sequenced. Association analysis of sequence variation in these genes and protein digestibility showed clear association but not strong enough suggesting that other factors may be confounding with PI to affect protein digestibility in sorghum.

Table 1. Cycsteine proteinase inhibitor proteins (genes) identified from sorghum flour samples using

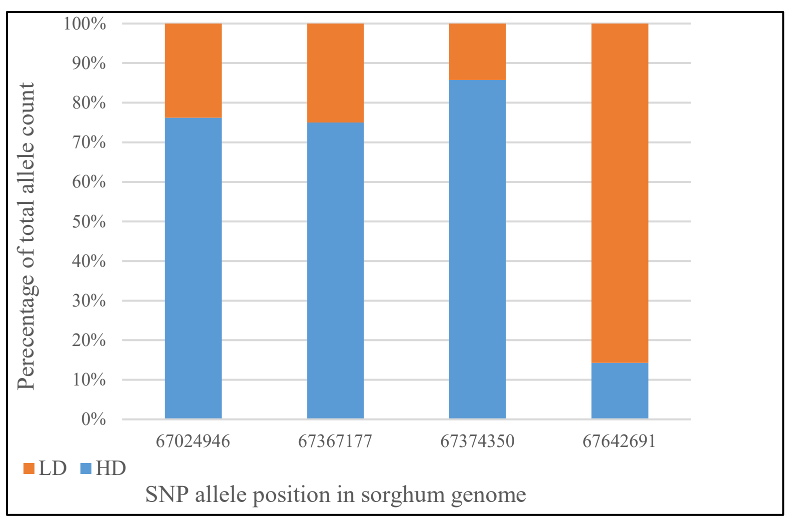
Gene ID	Genomic location	Description
Sobic.001G324500	Chr01:61170069..61170647 forward	Putativecysteine proteinase inhibitor 7
Sobic.001G324700	Chr01:61198278..61199489 forward	CYSTATIN FAMILY MEMBER
Sobic.001G324800	Chr01:61201114..61201921 forward	Cysteine proteinase inhibitor 8
Sobic.001G487800	Chr01:75769351..75770240 forward	CYSTATIN FAMILY MEMBER
Sobic.003G126800	Chr03:11616309..11619063 reverse	cysteine proteinase inhibitor 12
Sobic.003G400400	Chr03:70901405..70902262 forward	cysteine proteinase inhibitor 4

Because the identification of the protease inhibitor was delayed by about 2 years, there was not enough time to use these markers to screen the 2000+ materials as planned and identify variants with low PI activity. Instead, we carried out series of activities to explore the level diversity in sorghum storage protein, kafirin, across genotypes and identify natural kafirin alleles that are associated with improved protein digestibility.

The study revealed significant variation in kafirin gene sequences which is perhaps responsible for broad variation in both protein content and protein digestibility. The figure below shows the structure of the variation in kafirin genes which showed the beta (blue) gamma (red) and delta (green) kafirins occurring together occupying a smaller branch a cluster that contained several alpha kafirins. Two more clusters containing four and three alpha kafirin genes all from chromosome five occurred as separate branches (see figure below).



Additional activities were carried out to discover natural variants of kafrin alleles that are associated with improved protein digestibility. Since sorghum protein digestibility range from as low as 10% to a high of 80%, there appears to be several factors accounting for the traits and the property of the kafirins themselves may be among the major ones. This activity focused on identification of novel kafirin alleles that may be associated with protein digestibility and the result was very outstanding. Mining of nucleotide variation between kafirin genes of 57 sorghum genotypes representing high and low protein digestible groups was conducted. About 170 multiplex primer sets were synthesized to selectively amplify the entire kafirin gene clusters in all of the 57 genotypes. The amplicons were sequenced and bioinformatics analysis of the sequence data revealed four kafirin alleles (SORBI_3005G185600, SORBI_3005G188800, SORBI_3005G189000, and SORBI_3005G192801) that carry SNPs strongly associated with protein digestibility, three of them positively and the one negatively (see figure below). The three positive SNP alleles (the large blue bars) had missense mutation that resulted in change of amino acids from polar to hydrophobic side chains while the fourth allele that was associated with poor protein digestibility had mutation that caused change of amino acid from one with hydrophobic to polar side chain. One of the favorable genes is located on chromosome 5 of sorghum genome while the other two favorable genes and the unfavorable genes all located on chromosome 6. The unfavorable gene (large orange bar) was located far away from the favorable genes that linkage between the two will not be an issue. The graph to the right shows the distribution of the alleles among high and low digestible groups.



Analysis of sequence homology of these alleles with that of maize showed that alleles detected to be associated with improved protein digestibility had amino acid sequence change from that of like sorghum to one identical to maize showing that these alleles are indeed associated with protein digestibility. Once validated, these markers will become the first set of tools to be identified for use in breeding programs to improve protein digestibility. To realize that an optimized assay protocol suitable for routine screening of large breeding materials is needed. The Kompetitive Allele Specific PCR (KASP) assay system is a convenient and cost effective technological platform for accomplishing this.

The table below shows the property of all of the kafirin genes studied. Favaourable alleles are marked with green fonts and the unfavorable allele with red font.

Kafirin gene sequences and their genomic locations (extracted from the USDOE Joint Genome Institute, http://phytozome.jgi.doe.gov).

Gene ID	Kafirin subclass	Gene length (bp)	No. of exons	Amino acid sequence length (bp)	Genomic location
Sobic.005G188800	α	**943**	**1**	**269**	**Chr05:67366389…67367331**
Sobic.005G193180	α	951	1	269	Chr05:67661336…67662286
Sobic.005G184500	α	831	1	236	Chr05:66926726…66927556
Sobic.005G193000	α	947	1	268	Chr05:67648393…67649339
Sobic.005G193100	α	867	1	268	Chr05:67654898…67655764
Sobic.005G189000	α	**965**	**1**	**271**	**Chr05:67373729…67374693**
Sobic.005G192900	α	935	1	269	Chr05:67651628…67652562
Sobic.005G185400	α	998	1	288	Chr05:67014861…67015858
Sobic.005G184400	α	822	1	228	Chr05:66923278…66924099
Sobic.005G192801	α	**899**	**1**	**268**	**Chr05:67641925…67642823**
Sobic.005G184800	α	926	1	267	Chr05:66970093...66971018
Sobic.005G184600	α	711	2	183	Chr05:66931562…66932272
Sobic.005G186250	α	267	1	88	Chr05:67093967…67094233
Sobic.005G185600	α	**997**	**1**	**288**	**Chr05:67024041…67025037**
Sobic.005G186300	α	804	2	230	Chr05:67096608…67097411
Sobic.005G192700	α	905	1	268	Chr05:67638681…67639585
Sobic.005G193700	α	798	2	256	Chr05:67713094…67713891
Sobic.005G192901	α	895	2	266	Chr05:67645163…67646057
Sobic.005G193140	α	807	1	268	Chr05:67658193…67658999
Sobic.005G193220	α	949	1	268	Chr05:67664574…67665522
Sobic.005G184700	α	930	1	267	Chr05:66965527…66966456
Sobic.005G193260	α	899	1	268	Chr05:67667809…67668707
Sobic.008G144201	α	434	2	94	Chr08:57567069…57567502
Sobic.009G001600	β	788	1	192	Chr09:166827…167614
Sobic.010G136100	δ	351	1	116	Chr10:20530171…20530521
Sobic.002G211700	γ	872	1	212	Chr02:60423442…60424313
Sobic.002G211501	γ	978	2	230	Chr02:60415672…60416649

Allele count of SNPs with significant allele count difference between high (HD) and low (LD) grain protein digestibility sorghums. Chi-Square tests results for the SNP allele count differences between high and low digestibility sorghum panels were 67024946 ($\chi2$ = 6.85, p = 0.008) 67367177 ($\chi2$ = 3.75, p= 0.052), 67374350 ($\chi2$ = 6.85, p = 0.008), and 6764291($\chi2$ = 3.42, p= 0.064).

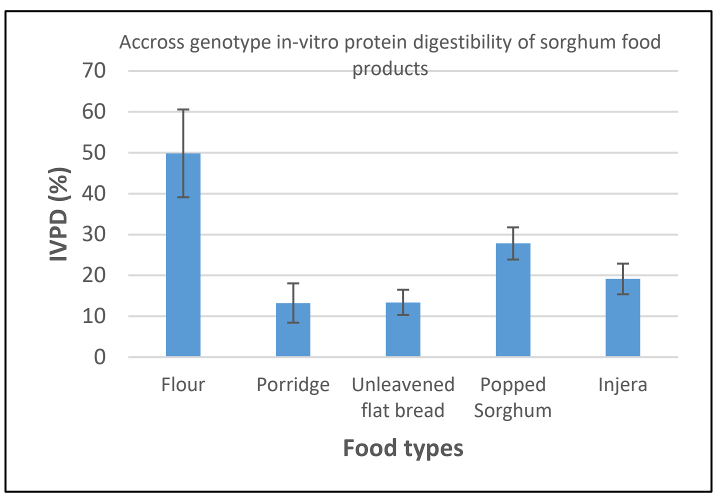
Obj. 2.3. Developing sorghum varieties/hybrids with superior yield potential, agronomic adaptation and enhanced nutritional value

Germplasm screening for nutritional traits (Obj 1.2 and 2.1) produced new breeding lines with enhanced protein content [PR11/12-824 (17% protein) and PR11/12-867 (19% protein)], and high protein digestibility [PR11/12-519 (72% protein digestibility) and PR11/12-525 (78% protein digestibility)]. On average, these lines contain 40-60% higher protein and also have 20 to 30% higher protein digestibility than average normal sorghum. Cultivar improvement effort was initiated following this discovery. Crosses were made between these lines and tropically adapted cultivars such as Macia and Dorado and between high protein content and high protein digestible sources. Segregating families were evaluated at Manhattan for agronomic potential and Puerto Rico for tropical adaptation. About 600 selected F3 families were sent to Ethiopian Institute of Agricultural Research (EIAR), Ethiopia for evaluation in 2017 and additional F4 families to Haramaya, Ethiopia in 2018. Currently field evaluation is underway at both Melkassa and Haramaya. The PI is currently on a sabbatical at Haramaya University and is closely working with these materials along with colleagues at the University. Many of the families show excellent adaptation to the new condition in Ethiopia that, despite the very low night temperature, are effectively growing, shedding pollen and setting seeds. Selected families are being included in the crossing block with seed parents introduced from both Kansas State and Purdue universities. Some of the females that are known to produce outstanding hybrids in Kansas such A/B527, A/B531 and A/B553 appear to have excellent adaptation to Ethiopia as lines per se and the performance of their hybrids is yet be seen next season. It is expected that varieties selected from this effort will have comparable agronomic performance with existing varieties and also have up to 20% higher protein content and 10% higher protein digestibility than the normal sorghum varieties grown in the country.

Moreover, multiple breeding populations were generated at Melkassa Research center of EIAR, Ethiopia between known high digestible local landrace (*Wotet be-gunchie*) and series of locally adapted released varieties. The EIAR team took charge of selecting and promoting superior families from the population. The F5 families derived from these populations have entered preliminary yield trial in 2017 season. Lines that express superior agronomic performance will be tested for protein digestibility and those combining the two traits will be advanced for further yield testing.

Objective 3: Optimize traditional sorghum food processing methods and for increased availability of proteins and calories in sorghum based diets

Obj. 3.1. Evaluate the effect of variety and traditional food processing on PI activity, anti-nutrient levels, and digestibility of proteins and starch in sorghum



Both variety and food processing methods had significant effect on protein digestibility and the concentration of some anti-nutritional factors. Evaluation of protein digestibility in four Ethiopian traditional food products (fermented bread, unfermented bread, porridge and roasted grain) from fifteen varieties widely grown in Ethiopia showed significant variation between genotypes. Protein digestibility from raw flour was highest for each genotype and across genotype followed by dry roast confirming the previous findings that wet cooking reduces protein digestibility. Nevertheless, there is significant genotype by food process interaction for protein digestibility as there is for bread quality. The Ethiopian high lysine cultivar

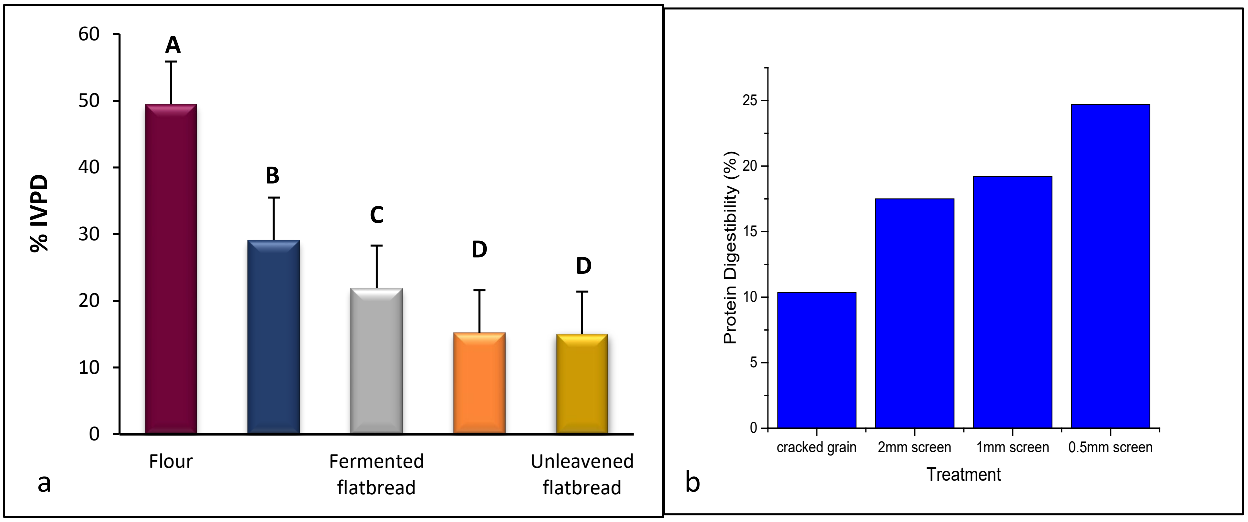
Wotet be-gunchie that had significantly higher protein digestibility in raw samples had low digestibility when cooked with digestibility values in dry roasted (popped) product and fermented bread was comparable with most varieties included in the test (see table below). Such interaction suggests that certain varieties are more suited for making certain type of product.

In-vitro protein digestibility of sorghum food products prepared from 15 diverse sorghum varieties.

	In-vitro protein digestibility				
genotypes	Flour	Porridge	Unleavened flat bread	Popped Sorghum	Fermented bread
IS 9302	38.80	11.50	12.40	31.80	23.50
Masugi Yellow	60.90	12.80	13.70	30.90	15.50
Teshale	40.70	16.60	12.10	31.50	20.80
Degalite Yellow	63.00	12.50	14.80	24.10	21.60
Jiguitte	56.80	10.70	13.80	29.80	22.20
OSMI 5064	49.90	18.50	19.10	28.20	16.10
Wetete-Begundi	75.10	26.60	19.10	29.30	17.80
Dagim	49.60	11.80	15.00	30.40	16.80
DESV 92021 DL	38.20	7.20	13.70	25.80	21.40
Meko	40.50	11.50	9.60	29.20	24.40
76T#123	44.60	13.70	13.20	25.30	19.20
Seredo	44.60	5.90	6.30	20.90	10.60
AL-70	50.20	14.80	14.20	23.90	14.10
Melkam	36.20	9.10	12.60	21.30	20.50
Chiro	58.60	15.40	11.50	34.80	22.40

Obj. 3.2. Optimization of sorghum food processing techniques to enhance protein digestibility

Traditional food processing methods were shown to have significant difference in in-vitro protein digestibility (see figure below). All food processes reduce digestibility with unfermented foods such as porridge and unleavened bread particularly having the least digestibility. Fermented bread (*injera*) commonly consumed in Ethiopia is less affected by cooking perhaps due to partial breakdown of starch by fermentative enzymes to free proteins, due to the acidic environment created as a result of fermentation or due to its effect on phytic acid and activation of phytase. In addition to food processes there is significant variety effect on both raw and cooked protein digestibility with very little correlation between them. A cultivar with high raw flour digestibility may not have high cooked digestibility. The digestibility of the high protein digestible Ethiopian landrace was found to be not significantly different from others when cooked to various foods. Future research aimed at addressing sorghum protein digestibility problem for human food need to focus on cooked products than raw samples. Further tests conducted to optimize *injera* making process showed both fermentation period and flour particle size have significant effect. Smaller particle sizes (< 0.5mm) increase protein digestibility, but it is not clear whether smallholder farmers have access to such a milling facility in the rural areas (see figure below). An expanded test is going on to see the effect of grain pre-processing such as malting, decortication, and semi-roasting on digestibility of cooked products, especially *injera*.



Protein digestibility of sorghum is significantly different between various food products (a) and for varying flour particle sizes for all genotypes (b).

Obj. 3.3. Promoting varietal technologies, and improved food processing methods for improved production and utilization

This outreach activity is planned to be executed in a form of training workshop in two regions of Ethiopia (the north and the east). The activity will bring together mainly female farmers in the selected regions since most of the food processing task is left to women. Male farmers are also invited to attend the training and appreciate one of the most important but rarely acknowledged problem of sorghum, its protein nutrition. In collaboration with the regional agricultural research institutes and the zonal agriculture offices, the Ethiopian Institute of Agricultural Research is organizing a training workshop in northern and eastern Ethiopia to be held in November 2018. In this training the results from food process optimization study will be presented and women farmers are encouraged to adopt the modified methods, using finely ground flour and giving enough time for fermentation to occur before breads are cooked. Relevant stakeholders from the Ethiopian nutrition institute and food science experts from regional universities will be invited to attend.

B. Major challenges encountered and resulting project adjustments

Challenges were met at different times in the project implementation period. But only one cause adjustment/modification to the activities. This was related objective 2.2. that focused on identification of protease inhibitor genes. Due to problems associated with purification of the samples, the identification of protease inhibitor genes took longer time than expected. The activity was completed two years after the planed completion date. As a result, although markers were developed to selectively amplify the six protease inhibitor genes identified, the markers could not be converted in to a form that will help screen large set of genotypes for variants of low protease inhibitor. Instead, another activity that focused on characterization of kafirin genes and finally led to the discovery of four novel alpha kafirin alleles associated with protein digestibility was performed. The delay in the hiring of postdoc caused by slow process of identification of PI genes, resulted in carry over of funds to next project period which was not viewed positively by the SMIL administration.

Other challenges are related to lack of timely requirement of graduate student which was delayed due to potential candidates not meeting graduate admission requirements, the slow process to acquire the 2000+ collections for nutritional quality analysis are worth mentioning. The other problem is the low throughput nature of the *in-vitro* protein digestibility assay. Collaborators in Ethiopia also faced certain challenges primarily due to lack of sufficient field supplies. This include pollination bags, aprons, hybridization kits, etc. Despite generous budget allocated for these, supplies have not become available primarily due to lack of vendors supplying the items. Other expected challenges are those related to weather conditions which complicated the implementation of the experiments in some of the locations.

C. Student training achievements

Two students were recruited to pursue graduate degrees in relation to this project. The first student (Yemane Belayneh) joined Kansas State University in December 2015 as PhD student. His research is focused on the development of genomic tools to assist breeding for nutritional quality. Nutritional parameters are unique in that, the traits are hidden from breeders' eyes and expensive and time consuming laboratory assay is needed to phenotype them. This has significantly reduced progress for development of cultivars with enhanced nutritional quality. Development and validation of genomic prediction models for use in genomic selection will minimize this problem and facilitate breeding efforts to improve the traits. Yemane's work is well in progress in this line and he is expected to complete his study by December 2019.

The other student (Alemnesh Bekele) was enrolled in MS program at Haramaya University in eastern Ethiopia. Her thesis research emphasized on analysis of nutritional quality of sorghum accessions from eastern Ethiopia. Alemnesh has generated series of data on both grain and nutritional quality parameters from about 500 sorghum accessions which included analysis of both major and minor nutrients. The study identified series of accessions with exceptionally high iron content and also of zinc. Alemnesh has successfully completed her degree and is currently in charge of the sorghum breeding program at Haramaya University.

D. Short-term training and outreach

Short term training is planned for small holder women farmers to communicate the results of the food process optimization activity. In collaboration with the regional agricultural research institutes and the zonal agriculture offices, the Ethiopian Institute of Agricultural Research is organizing a training workshop in northern and eastern Ethiopia to be held in December 2018.

6 – UTILIZATION OF RESEARCH OUTPUTS

A. Technologies and management practices by phase of development

Activities related to objectives 1 and 2 produced intermediate outputs on which next activities can be based to develop finished technologies. Objective 3, on the other hand, generated significant outputs that can be directly used by small holder sorghum consumers. Optimization of injera (fermented bread) processing method, extended fermentation and smaller flour particle size significantly contributed to improved protein digestibility. The improvement in digestibility appear to come from the effect of fermentation on anti-nutritional factors such as phytic acid that was shown to be significantly lower in fermented products compared uncooked flour. Another ongoing research which is set to determine the impacts of decortication, sprouting and roasting along with extended fermentation and smaller flour particles is expected to contribute to further improvement of protein digestibility. Data on the latest activity is expected to become available by the end of the year.

B. Intermediate outputs

The first phase of the project has generated significant intermediate outputs that will serve as stepping ground for next activities. Results of these intermediate outputs have been presented in conferences and are being published in international journals. Some of the outstanding intermediate results of the project include:

1. The assembly and characterization of 2000+ sorghum germplasm collections of Ethiopian origin

2. The identification high protein content (19%) and high protein digestible (78%) lines

3. The identification of protease inhibitor genes

4. The discovery of novel alpha kafirin alleles associated with protein digestibility

5. The development marker systems to selectively amplify the genes

6. The development or robust breeding population and families involving parental sources of high yield potential, high protein content and high protein digestibility

7. The development of improved food (*injera*) processing methods for enhancing protein digestibility and their effect on anti-nutritional factors

C. Publications

Dilooshii Weerasooria, Scott Bean, Yohannes Nugusu, Brian Ioerger, and Tesfaye Tesso. 2018. The effect of genotype and traditional food processing methods on in-vitro protein digestibility (IVPD) and micronutrient profile of sorghum cooked products. Plos One. https://doi.org/10.1371/journal.pone.0203005

Dechassa Duressa, Scott Bean, Michel Tilley, and Tesfaye Tesso. 2018. Genetic basis of protein digestibility in grain sorghum [sorghum bicolor (l.) Moench]. Crop Science 58:2183-2199.

Conference presentations

Dilooshii Weerasooria, Scott Bean, and Tesfaye Tesso. 2018. The impact of genotype, food processing methods and kafirin composition on digestibility of sorghum proteins. Conference presentation, Sorghum in the 21st Century, Cape Town, South Africa, 9-12 April, 2018.

Yemane Belayneh, Geoffrey Morris, Sanzhen Liu, Taye Tadesse, Amare Seyoum; Getachew ayana, Daniel Nadew, Alemnesh Bekele, and Tesfaye Tesso. 2018. The dynamics of tannin presence in Ethiopian sorghum landraces follows climatic cues. Conference presentation, Sorghum in the 21st Century, Cape Town, South Africa, 9-12 April, 2018.

Alemnesh Bekele, Firew Mekbib, and Tesfaye Tesso. 2018. Genetic variability and characters association of Hararghe sorghum [Sorghum bicolor (l.) Moench] genotypes for grain yield, yield components, grain and nutritional quality. Conference presentation, Sorghum in the 21st Century, Cape Town, South Africa, 9-12 April, 2018.

Dechassa Duressa, Scott Bean, Paul St. Amand, and Tesfaye Tesso. 2018. Association Between Kafirin Allelic Variants and Sorghum Grain Protein Digestibility. Conference presentation, Sorghum in the 21st Century, Cape Town, South Africa, 9-12 April, 2018.

Tesfaye Tesso. 2018. Integrated approach for enhancing productivity and nutrition for smallholder sorghum producers. Conference presentation, Sorghum in the 21st Century, Cape Town, South Africa, 9-12 April, 2018.

Dilooshi Weerasooriya, Scott Bean, and Tesfaye Tesso. 2017. In-vitro Protein Digestibility, Phytic Acid and Phytase Concentrations in Sorghum as Affected by Genotype and Food Processing Methods. Agronomy Society of America, Crop Science Society of America and Soil Science Society of America, 22-25 October 2017, Tampa FL.

Alemnesh Bekele, Firew Mekbib, Ketema Belete and Tesfaye Tesso. 2017. Genetic Variability and Characters Association of Sorghum Germplasm Collections from Eastern Oromia Region of Ethiopia. Agronomy Society of America, Crop Science Society of America and Soil Science Society of America, 22-25 October 2017, Tampa FL.

Publications in review

Dechassa Duressa, Scott Bean, Paul St. Amand, and Tesfaye Tesso. 2018. Identification of α-Kafirin Alleles Associated with Protein Digestibility in Grain Sorghum [Sorghum bicolor (L.) Moench]. The Plant genome (Under Review).

Gezahegn G. Tessema, Habte Nida, Tesfaye Tesso, Gebisa Ejeta, Tesfaye Mengiste. 2018. A Large-Scale Genome Wide Association Analyses of Ethiopian Sorghum Landrace Collection Reveal Loci Associated with Important Traits. BMC Genomics (Under review).

Thesis publications

Alemnesh Bekele. 2018. Genetic variability and characters association of Hararghe sorghum [Sorghum bicolor (l.) Moench] genotypes for grain yield, yield components, grain and nutritional quality. Haramaya University, School of Graduate Studies (Completed and published June, 2018).

7 - STATEMENT ON PROPOSED FUTURE ACTIVITIES FOLLOWING PHASE I RESEARCH ACCOMPLISHMENTS

A. Statement on future activities in phase II

Future effort in improving nutritional quality of sorghums based food products should combine information from results of the current study and other similar studies to help develop technologies and food processing practices that can alleviate the problem of protein nutrition in smallholder farmers.

1. *Analysis of sorghum nutritional quality using human subjects*: Most of the data available on nutritional quality especially protein digestibility of sorghum is based on in-vitro test results. While this is a good indicative, it does not fully represent the whole biological processes happening in human gut. This in-vitro test which involves digestion of samples for 2 hours with pepsin enzyme at certain pH does not take into account the basic structural and chemical differences between grains of different species. Recent findings showing significantly longer gastric emptying time of sorghum based food products not only indicate that the in-vitro assay does not show the whole picture but also suggests that protein nutrition of sorghum may not be as bad as what the in-vitro test results show. A discussion was initiated between the Ethiopian Nutrition Research Institute, Universities and EIAR/SMIL PIs to undertake a comprehensive human nutrition study that compares the nutritional outcome of sorghum, maize and other cereal food products using volunteer adult pupil from various universities. However, the study was not conducted due to lack of financial support. It is important that such study be conducted to determine the value of sorghum as human food and thus to justify future investments to improve the trait. This study will be relatively inexpensive and can be conducted in short period of time.

2. *Simultaneous improvement in protein content and protein digestibility* – Results from the current study showed that genotypes with higher protein content tend to have low percent protein digestibility than those with low percent protein both in cooked and uncooked state. However, estimation of the actual amount of protein digested from genotypes contrasting for percent protein digestibility show that the absolute amount of protein digested in both high and low digestible genotypes was not different. Thus given that there at least as much variability in protein content among genotypes as protein digestibility and the assay procedure is

even simpler for protein content, it is important that emphasis be placed on improving protein content and kafirin composition of sorghum in addition to increasing percent protein digestibility. Research through this phase of the project has identified series of genotypes with up to 19% protein content. We have initiated series of breeding populations involving high protein content and high protein digestible sources and about 600 F4 families derived from these populations are currently growing in Ethiopia. It is important that the most promising families be further advanced and phenotyped for protein content and digestibility. Some of the families have excellent pollinator parent characteristics that test cross hybrid synthesis based on these pollinators is going to produce hybrid seeds for agronomic evaluation.

3. *Exploiting the protease inhibitor and novel alpha kafirin variants to breed sorghum cultivars with low concentration of enzyme resistant kafirins and reduced PI activity* – Phase I of our project generated a ground breaking discovery on sorghum protein nutritional quality research. Six protease inhibitor genes and four alpha kafirin alleles were shown to have strong association with protein digestibility in sorghum. We have developed markers that can selectively amplify these alleles. The next research on this line will be to utilize these markers to screen the 2000+ germplasm collections from Phase I for variants of the PI genes and the four alpha kafirin alleles in order to identify sources with low PI activity and low concentration of enzyme resistant kafirins. The current work also showed gamma kafirin as having significant and negative effect on protein digestibility. In the next phase, we plan to use the information on both protease inhibitor and gamma and alpha kafirin genes to select sources with low PI and desired alpha kafirin alleles and combine those with high protein sources and tan plant germplasm with little or no anti-nutritional factors to develop nutritious agronomically desirable sorghum varieties for smallholder producers. In preparation for this, besides the 600 high protein content and protein digestible families currently under evaluation in Ethiopia, new breeding population involving these sources and series of varieties adapted to the Ethiopian condition is currently underway. These populations will be subjected to agronomic evaluation, genotyping for the novel alpha kafirins discovered in phase I of this project and will also be subjected to genomic selection for enhancing both protein content and digestibility.

4. *Integrating nutritious variety/hybrid development activity with traditional cropping systems* – This activity was proposed in phase I of the project as self-standing objective but was later dropped due to funding limitation. The traditional practices of small holder farmers where multiple crops are cultivated on the same field at the same time is a common practice. Especially in sorghum producing regions of eastern Ethiopia, this is routinely practiced. In addition to increasing production per unit area, the practice contributes to food and nutritional security through food diversification and ensuring sustainability. We believe that superimposing the cropping system practice on to the high protein and high protein digestible variety development effort will result positive outcomes. Cereals like sorghum are often intercropped with legumes, especially haricot bean which not only improves the overall productivity but also helps farmers get a high protein grain. Existing varieties and hybrids as well as experimental hybrids from our programs including the high protein high protein digestible materials as well as varieties of unique canopy architecture may be evaluated in various cropping systems and management practices. There is tremendous opportunity for developing varieties with unique plant architecture that reduces completion on component crops allows erect additional have crop diversity nutrition.

B. Linkage to Phase I objectives and activities

Activities 2 and 3 described above are directly related to the outcomes of the current project and thus is strongly related to Phase I activity. Activity 1 is a basic information that is needed to be done and it does not have to be done through phase II of this project. SMIL- ME can assign small resource to food scientists to have the activity performed with relatively low cost and in short period of time. Activity 4 which was also proposed in phase I proposal, reviewed positively but dropped due to shortage of funding is very critical in that it can address both the nutrition and productivity issues in much more sustainable and ecofriendly manner. It is not based on outputs of phase I project but on the reality and practices that farmers routinely implement and its potential for sustainability and more positive nutritional outcomes.

C. Training and outreach objectives

The key outreach activity in the next phase of the project should be on creation of awareness of local communities about the value of healthy nutrition and presenting them with practices, tools and technologies that help improve protein nutrition in sorghum based foods. Local farmers have little knowledge of nutritional value of foods they eat. Moreover, Ethiopia is expanding its educational and research institutions that capacity building through advanced degree training to staff these institutions is essential. SMIL has made remarkable achievement in its Phase I as did its predecessor, INTSORMIL, in the 30 years. Hence capacity building through graduate student training and mentoring and short term visits will continue to be the major training objective.

WEST AFRICA

IMPROVING SORGHUM ADAPTATION IN WEST AFRICA WITH GENOMICS-ENABLED BREEDING (SAWAGEN)

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3 - PROJECT GOALS AND OBJECTIVES

The project activities of phase-I addressed two overall goals in seven specific objectives:

Overall goals:

1. **Goal 1:** Develop human capacity (trainees and network) needed for genomics enabled-breeding
    a. Develop early-career scientists with fully supported PhD training of two geneticist and two breeders in genomics-enabled breeding, and other graduate students (described below, in Students Trained)
    b. Built an R&D network for West African NARS sorghum programs (Senegal, Niger, Togo; with Mali and Burkina Faso involved) that includes shared germplasm, collaborative publications, regular conference calls, and training exchanges.
2. **Goal 2:** Develop technical resources (data, tools, lines) needed for genomics-enabled breeding
    a. Development of all the technical resources needed for a genomics-enabled breeding program, including a diverse association panel, multiparent mapping populations, managed stress phenotyping capacity, genome-wide markers, and trait-predictive markers.

b. Initial deployment and testing of the genomics-enabled breeding system via marker assisted recurrent selection.

Specific Objectives:

- **Objective 1:** Genomic characterization of West African germplasm
- **Objective 2:** Build genomics-to-breeding toolkit
- **Objective 3:** Multi-parent population development
- **Objective 4:** Trait-mapping for grain mold, *Striga*, and drought
- **Objective 5:** Marker assisted recurrent selection and genomic selection
- **Objective 6:** Long- and short-term training
- **Objective 7:** Proactive gender integration in project activities

4 - OVERVIEW OF ACTIVITIES

Objective 1: Genomic characterization of West African germplasm

The aim of this objective was to understand the relationships among West African landraces and breeding programs, and to identify genomic regions contributing to adaptation. Data from this objective was also used for trait-mapping and genomics-to-breeding toolkit objectives, below. We conducted genotyping-by-sequencing (GBS) of more than 2,000 West African breeding lines and landraces, and analysed ~400,000 SNP markers. About 1,500 of these accession were obtained from the US genebank (GRIN), including complete national collections from Niger (N = 520, Maina et al. 2018), Nigeria (N = 541, Olalere et al. 2018), and Senegal, Gambia, and Mauritania (N = 421, Faye et al. in review). In addition, approximately 750 accessions from the West African Sorghum Association Panel (WASAP) have been genotyped with GBS.

Objective 2: Build genomics-to-breeding toolkit

The aim of this activity was to develop rapid and cost effective marker systems to enable sorghum molecular breeding in West Africa, converting GBS-to-KASP markers, when possible focusing in gene of agronomic interest, and use them for breeding purposes. In a first step, we used the results of the GWAS that were obtained using both the WASAP collection and the African germplasm that reside in the US collection, identifying SNP markers linked to yield component traits (grain weight and number) and to adaptive traits (stress tolerance index and dry zone adaptation). In a second step, we referred to the literature (Gobena et al., 2017) and contacted the PIs of on-going projects (Tuinstra SMIL project on protein digestibility, Deshpande ICRISAT project on stay-green) to identify SNP markers tightly linked to genes/loci controlling *Striga* resistance, protein digestibility and terminal drought tolerance.

Objective 3: Multi-parent population development

The aim of the activity was to develop multi-parent mapping populations that facilitate trait-mapping, marker development, and genomics-assisted breeding. We developed mini-Nested Association Mapping (mini-NAM) populations in Senegal and Niger.

Senegal mini-NAM: For the development of the mini-NAM populations we crossed Nganda (a recently released white seeded, high yielding ISRA variety) with Sureño, Macia (Caudatum-type mold resistance donors), and CSM-63E (Guinea-type, drought tolerant donor). We produced $F_{3:5}$ populations for trait mapping and for running the marker-assisted recurrent selection (MARS) approach, and three connected RIL populations.

Niger mini-NAM: To develop a biparental mapping populations and further develop new varieties with resistance to major stressors in Niger, a common parent (locally improved variety) MDK was crossed to resistant lines. This activity consisted in the constitution of a mini-NAM population by INRAN (Niger) using MDK x ICSV88032 (midge resistant line) cross which is at F3 (by WACCI student who ended his thesis in 2017) and make two more biparental RIL families with MDK x SRN39 (*striga* resistance) and MDK x L153-5 (early improved variety drought tolerant line). Currently, F6 population is being developed for the drought tolerance and *striga* resistance and F5 population for the midge resistance.

Objective 4: Trait-mapping for grain mold, Striga, and drought

Managed drought stress phenotyping – WASAP (ISRA/CERAAS)
The beginning of the project a West African Sorghum Association Panel was constituted with more than 600 accessions from the working diversity collections of Mali, Niger, Senegal, and Togo. The majority of the accessions are landraces. In experiments conducts over four years by Bassirou Sine, the WASAP was assessed into nine trials with three contrasting water regimes at CNRA-Bambey in Senegal:

1. One trial was conducted during the rainy season 2014 for seeds multiplication.

2. Three trials in well-watered (ETM) conditions during the hot offseason 2015, and cold off-season 2016 and 2017.

3. Three trials under pre-flowering water stress (STR1) during the hot off-season 2015, and cold off-season in 2015-2016 and 2016-2017.

4. Two trials under post-flowering water stress (STR2) during the cold off-season in 2015-2016 and 2016-2017.

During these trials, climatic parameters and fraction of transpirable soil water (FTSW) were measured to characterize environmental conditions. Phenotypic measurements included plant phenology (date to 50% emergence, flowering and maturity), morphology (plant height, stay green and lodging, leaf number, stem number, number and weight of panicles per plant), yield components (aerial dry biomass, 1000-seeds weight, harvest index, grain weight and grain number per plant) and physiology (chlorophyll fluorescence, leaf temperature). The drought susceptibility index and the Stress Tolerance Index (STI) were calculated using grain weight per plant from well-watered and water-stressed trials. The phenotypes were used in GWAS to map loci controlling water-limited yield components. In total, we identified 56 SNPs associated with BLUP phenotypes across all experiments and 26 SNPs associated with STI (results based on multi-locus mixed model).

Multi-environment grain mold phenotyping – Senegal mini-NAM (ISRA/CNRA)
Phenotyping experiments were undertaken during the rainy season 2015 and 2016 in three ISRA research stations (Bambey, Darou and Sinthiou Malem) totaling six environments. In each environment, 600 F3:5 families of the mini-NAM populations were installed in an augmented experimental design with 40 blocks of 19 entries each. Twelve traits were quantified: time to 50% flowering (TFlo), plant height (PH), peduncle length (LPed), panicle length (LPan), panicle width (WPan), panicle weight per plant (PanWP), grain weight per plant (GWP), thousand-grain weight (TGW), number of grain per panicle (NGP), texture of endosperm (TEnd), panicle and threshed grain mold rating (PGMR, TGMR).

Managed Striga stress phenotyping – Reference lines (INRAN)
The first activity was *striga* collection. The collection was done in Tahoua region, one of the most infested regions of the country, where 12 villages were visited in November 2014. In May 2014, fifty (50) accessions of *striga* resistant lines were received from Ethiopia. Seed increase was undertaking at the INRAN station at Maradi during the raining season. *Striga* samples were used frequently to evaluate ten (10) Ethiopian lines and five (5) local varieties (MDK, L153-5, SRN39, Mota Maradi, and El tsedaoua) at Konni research station to prevent infestation in other research stations. The screening was performed over three years during rainy season. Besides phenotypic observations, the level of infestation and emergence of *striga* were recorded (Seedling vigor, *striga* emergence, number *striga* at 45-, 60-, 90-days, and at harvest, 50% flowering, plant height, panicle weight, and yield).

Managed water stress phenotyping – Test lines (INRAN)
To evaluate local varieties under water-limited conditions, and build phenotyping capacity for drought stress, we screened 20 lines (McKnight project's P tolerant varieties) using split plot design with five replications (water regime as main plot and varieties as split plot).

Lysimeter drought stress phenotyping – WASAP (ICRISAT)
To identify traits underlying drought tolerance, phenotyping of sorghum landraces from Niger and the West African Sorghum Association Panel (WASAP) was conducted under managed drought stress in a lysimeter system. The pre-flowering and post-flowering drought stress were imposed by limiting irrigation. Five (5) experiments were conducted in the lysimetric system at ICRISAT Sadore to assess the sorghum durra landraces from Niger (10 lines) along with checks (2015 and 2016 in rainy and post-rainy season) and the WASAP along with checks under pre-flowering (rainy seasons 2017 and 2018, ongoing). The WASAP experiments aimed to (i) understand the mechanism of drought stress, (ii) perform GWAS for agro-morphological traits, (iii) evaluate the found QTLs compared to the GWAS in field experiment and (iv) identify QTLs that contribute to drought tolerance useful for marker assisted breeding.

Objective 5: Marker assisted recurrent selection (MARS) and genomic selection (GS)

The aim of this activity was to begin implementation and evaluation of two potential genomics-enabled breeding (GEB) approaches, MARS and GS. This began with genotyping of NAM populations and trait-mapping. Polymorphism between parental lines (i.e. Nganda vs Sureño, Macia and CSM-63E) as well as other elite lines of ISRA breeding program was first surveyed using the 1400 KASP-SNP markers that were developed under the framework of the GCP. In total, 24 lines were assessed for polymorphism. We selected a subset of 201 SNP markers to genotype the mini-NAM $F_{2:3}$ populations based on these criteria: (i) the polymorphism between Nganda and all three parental lines, (ii) the position on the sorghum physical map that offers the best possible coverage, (iii) the polymorphism between Nganda and at least two parents of the populations. These SNP were used to genotype the mini-NAM (F3:5). QTL mapping was performed using genotypic and phenotypic data from Nganda x Sureño offspring. To generate high-density reference genotyping data, DNA from F6 lines has been collected and sent to KSU for genotyping.

Objective 6: Conduct long- and short-term training

The long-term training focused on PhD training in the US for West African geneticists and PhD training in WA for West African breeders. Short-term scientific training aimed to introduce West African graduate students to genomics-enabled breeding. Short-term practical training aimed to introduce farmers to improved seed production techniques and varieties under development by NARS.

Objective 7: Proactive gender integration in project activities

Here we took proactive steps to take into account gender-specific constraints, preferences and practices in project activities in order to assure equitable participation and input from male and female farmers, processors and consumers. These aims were implemented in (i) recruitment of long- and short-term trainees, (ii) participatory rural appraisals, and (iii) participatory varietal selection.

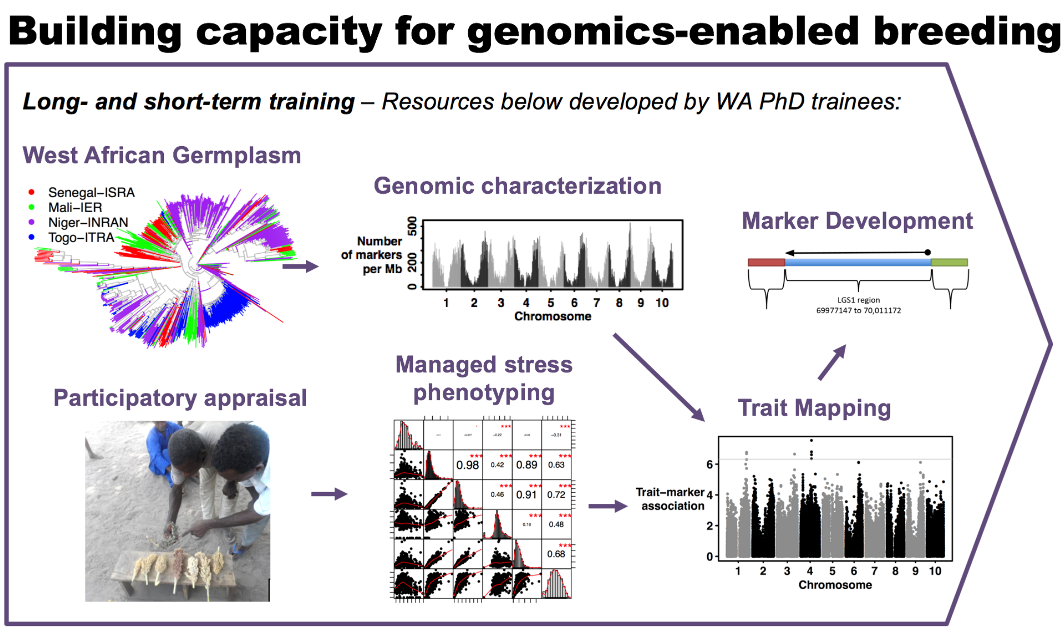

FIG. 1: **In phase-I we developed human capacity and technical resources for genomics-enabled breeding in West African NARS.** A generalized workflow for development of capacity and resources is shown. The exact order depends on the starting capacity and resources. The long-term PhD trainees developed genomics, phenotyping, and participatory knowledge and resources that they will deploy in their own genomics-enabled breeding programs.

5 - ACCOMPLISHMENTS

A. Achievements by project objectives

Objective 1.1: Genotyping-by-Sequencing SNP map for West African germplasm

We have completed genotyping at 400,000 markers for over 2000 West African sorghum accessions. The data set allowed us to precisely understand the relationships among West African national landrace collection and breeding populations (Maina et al. 2018, Olatoye et al. 2018, Faye et al. in review). The identification of diagnostic markers to recover local genetic backgrounds is ongoing. These genomics resources are now available to the global sorghum improvement community with raw sequencing data were deposited to the NCBI Short Read Archive and genotype data available at Dryad Digital Repository.

Objective 1.2 Identification of adaptive genomic regions in West African germplasm

Completed for Nigerien, Senegalese, and Nigerian germplasm representing over 1,500 West African accessions. We identified 1191, 829, and 144 candidate adaptive genomic regions that had been under selection in the Senegalese, Nigerien, and Nigerian sorghum accessions, respectively.

Objective 2.1 Release of open-source GBS-to-KASP conversion tools

GBS-to-KASP conversion is underway for ~100 markers. Development of conversion tools themselves are on hold, pending further development of (i) high-throughput genotyping databases (GOBII, GIGWA) that provide easy access to genomic data and (ii) MAS capabilities in breeder-facing decision support software (BMS or equivalent). A total of 97 SNP-KASP markers were developed. These SNP tag genes involved in maintaining grain weight and number under intermittent or terminal water deficit conditions, in stress tolerance indices, in panicle length, in *Striga* resistance, in protein digestibility, and stay-green trait.

Objective 3.1 Develop Senegal mini-NAM population

The Senegal mini-NAM has been advanced to the F7 generation by SSD in Bambey with the number of offspring per family is indicated below.

Population	Population Name	Generation	Number of families
Nganda x CSM63	NGAC	F7	200
Nganda x Macia	NGAM	F7	180
Nganda x Sureño	NGAS	F7	220
TOTAL	-	-	600

Objective 3.2 Develop Niger mini-NAM population

The Niger mini-NAM families have been advanced to the F6 generation in Maradi and Tillabery. In 2017, we obtained 262 F5 (MDK x L153-5) and 77 F5 (MDK x SRN39) in Tillabery and 150 F5 (MDK x L153-5) and 55 F5 (MDK x SRN39) in Maradi. Pedigree selection was used for the choice of F_4 lines through participatory approach. Breeders, Halal seed Company and farmers were invited in each site to make choice. Fifty other F_4 were advanced through this method with farmers and in station in the bid to come out with variety which fitted with farmers need.

Objective 4.1 Phenotypes for West African sorghum mapping populations

Field phenotyping under managed drought stress (CERAAS)
A phenotypic data base is constituted with data from the six trials during the off seasons 2015-2016 and 2016-2017. The fraction of transpirable soil water (FTSW) and response of international check lines (B35 and Tx7000) indicated that the targeted drought stress was reached for each trial. Pre-flowering drought stress delayed flowering time and post-flowering stress accelerated maturity. This study highlighted grain yield potential related traits under pre- and post-flowering drought conditions. Eight promising accessions have been identified as a sources of tolerance for improving water deficit adaptation in West Africa. These local sources of stress tolerance will be an advantage for varietal improvement in West Africa where farmers prefer their local varieties.

Lysimeter phenotyping under managed drought stress (ICRISAT)
The assessment of sorghum landraces from Niger in lysimeter system under drought conditions in pre-rainy rainy and post-rainy seasons showed a genetic variation in days to flowering for local landraces. After imposing post flowering drought stress for 26 days, large variation was observed on the total extracted water per plant, indicating significant differences in water requirement among the accession. Low transpiration led to high seed weight and revealed associated with pre-flowering drought tolerance. Several lines showed low transpiration under drought stress (B35, CSM63, Dawa makefo, El Macika, Fara dawa, and IRAT 204) indicating drought avoidance strategy to conserve water, compared to Togolese material which showed high transpiration due to

low stomatal adjustment. The high genotypic variation in panicle weight under drought conditions suggests contrasting materials useful in breeding programs.

Objective 4.2 Trait-associated markers for grain mold, *Striga*, and drought

Grain mold in Senegal mini-NAM

A total of 600 F3:5 individuals were produced from the crosses between Nganda and three donor parents (Sureño, Macia and CSM-63E). The Nganda x Sureño family was used for QTL mapping, with 32 F_3 families showed low grain mold incidence in 2–3 environments. Overall, 30 QTL were mapped when considering all environments and years. Except for plant height QTLs, all detected QTLs have small effects ranging from 0.1% to 3.7%. Interestingly, on SBI-03, the map position of QTL for TGMR coincided with the one of QTLs for panicle length, panicle width, and thousand grain weights. Sureño contributed favorable alleles at all grain mold resistance QTLs while Nganda contributed favorable alleles at all yield components QTLs.

WASAP lysimeter drought response

Lysimeter phenotypes under pre-flowering drought stress from ICRISAT were used to perform GWAS at KSU. GWAS was conducted on 189 genotypes for the 2017 experiment. The traits associated markers identification with GWAS revealed 7 significant SNP markers associated with plant height, panicle and grain weight.

Objective 5.1 Develop local germplasm with improved resistance traits with MARS

MARS was not implemented given that at all QTLs for yield component and phenological traits, the favorable alleles were from Nganda and that few mold resistance QTL with very small effects were detected. Nevertheless, we identified some F4 individuals that accumulate the Sureño alleles at the four grain mold resistance QTLs. These lines, now at F6 generation, will be evaluated for resistance.

Objective 5.2 Evaluation of genomic selection models for West African breeding

Based on the genomic analysis, we have determined that the current genetic structure of WA breeding programs is not conducive to standard genomic prediction models (insufficient kinship). In the SMIL-Haiti project we are currently evaluating approaches to account for low kinship and large effect polymorphism in developing country breeding programs. Based on these findings, PhD trainees Faye and Maina will begin cross-validation analyses with appropriate genomic prediction models in late 2018.

Objective 6.1 Long term training in genomics-enabled breeding

Two PhD trainees at KSU (Fanna Maina, Niger; Jacques Faye, Senegal) completed English language training, entered the PhD program, and advanced to candidacy. An additional KSU-funded PhD student (Marcus Olatoye, Nigeria) completed his PhD based in part on SMIL genomic data. First-author manuscripts have been published/submitted for all KSU students. Two PhD at CERAAS (Cyril Diatta, Senegal; Eyanawa Akata; Togo) have completed their PhDs and are launching their GEB programs using phase-I resources. Additional training information is provided below, in "*c. Student training achievements*".

Objective 6.2 Short term training workshops on genomics tools and improved germplasm

We conducted multiple workshop/seminars in Senegal on genomics-enabled breeding. In addition, training on seed multiplication was provided to women and men farmers and seed producers. Details and additional activities are provided below, in "*d. Short-term training and outreach*".

Objective 7.1 Incorporate male vs. female preferences when deciding which local varieties to incorporate into populations crosses

Farmers participatory varietal selection
Gender consideration with female producers participated in the selection and/or training Tillabery and Maradi in Niger. Three lines from N13-derived lines from ICRISAT-Ethiopia ((AG-8XN13) BC3F5-32, (TXN13) BC3F5-41, and (AG-8XN13) BC3F5-42) were selected by farmers because of their resistance to *Striga*, their high yielding, and their adaptation. These varieties will be used to make crosses with the sorghum program best varieties and landraces to develop *striga* tolerant genotypes. During participatory selection, farmers and seed producers selected lines from the mini-NAM population at F4 generation.

Participatory Rural Appraisals in Senegal, Niger, and Burkina Faso
In Senegal, Cyril Diatta has consulted with women and men farmers in multiple regions to develop breeding priorities. Importantly, the PRA revealed that *Striga* resistance a high priority in the main sorghum growing regions. In Niger, Ousseini Ardaly surveyed farmers in Tahoua and Maradi regions on sorghum production, main constraints, and methods to reduce *striga* infestation. These findings, along with those shared by Nofou Ouedraogo, were synthesized collaboratively into country-specific product profiles developed by sorghum breeders at ISRA, INRAN, ITRA, and INERA.

Objective 7.2 Utilize key points of contact at universities and other institutions to identify qualified female candidates for long- and short-term training.

For long-term training, trained one female PhD (versus three male PhD) and two female MSc/Licence (versus one male). Details on short-term training are provided in a table below.

Communication and integration

One final achievement we would like to highlight, which is not represented in any individual objective, is the level of communication and integration we have attained through the project activities. For instance, in the past 2 years we met for 19 project conference calls, with up to 12 participants from up to 6 countries. We have also helped establish a multi-crop West African germplasm exchange/testing network, with two of our PIs coordinating the inaugural meeting and 5 project PIs/collaborators participating.

B. Major challenges encountered and resulting project adjustment

Linking francophone and anglophone research communities

- *Challenge:* The PhD trainees who were selected (from francophone countries) had limited English, but English is essential for US-based PhD training and/or publication in international journals.
- Pr*oject adjustment:* Full-time English language training opportunities and/or visits to U.S. were added to increase English proficiency.
- *Lesson learned:* English language training (formal and/or informal) is a major investment required so francophone West African scientists can participate fully in the global scientific community.

Data-driven versus hypothesis-driven applied science

- *Challenge:* The data-driven approach originally conceived for the project (e.g. large-scale genomics, phenotyping, etc) threatened to overwhelm PhD trainees and generate less impact than expected.

- *Project adjustment:* A goal-directed hypothesis-driven (GOHY) framework was developed, and project activities were reframed and refocused based on the framework. This meant reducing the data generated and increasing the time devoted to training on the scientific method.

- *Lesson learned:* A hypothesis-driven approach to applied research and training is more effective than a data-driven approach and should be implemented rigorously from project conception to completion. Hypothesis-driven studies create greater engagement across disciplines than descriptive studies.

Slower than expected development of software for NARS breeding

- *Challenge:* Our project initially intended to develop genomic-assisted breeding tools that would be deployed via IBP's Breeding Management System (BMS), but marker-assisted selection (MAS) functionality in BMS has not been developed as planned.

- *Project adjustment:* While development and deployment continues for BMS and other decision support software, we have been moving forward with a collaborative approach that does not depend on software.

- *Lesson learned:* Since decision support software for marker-assisted breeding may still be years away, we are developing a model of NARS breeders as "collaborator-clients" and a CERAAS hub as a MAS service and scientific support provider.

Photoperiod effects in managed stress phenotyping

- *Challenge:* Growing the WASAP in rainy season or hot offseason is problematic due to photoperiodic sensitivity of most of the accessions, which delays flowering and makes it more difficult to impose water stress at the same time.

- *Project adjustment:* Conducting trials during the short-day time in the cold offseason synchronized flowering times and facilitated consistent stress treatment.

- *Lesson learned:* Reference data on cycle length and photoperiod sensitivity should be used to plant genotypes at different dates in order to impose drought stress at the same time.

Environmental effects in grain mold phenotyping

- *Challenge:* Accurate phenotyping for grain mold resistance is challenging as the trait is highly influenced by the environment (e.g. north-south gradient in disease pressure, interaction with insect attacks), so heritability is low. This raises the questions for the strategy of deploying grain mold resistance in tannin-free, white seeded ISRA varieties.

- *Project adjustment:* A greater focus was placed on phenotyping and mapping of maturity and panicle morphology in the grain mold study.

- *Lesson learned:* The experiment highlighted the benefits of photoperiod sensitivity and open panicles for escaping grain mold. QTL for these traits should be used for adapting the ISRA sorghum varieties to the Senegalese agro-climatic conditions allowing them to mature out of the high humidity period and dry more readily.

Effectiveness of MARS for complex traits

- *Challenge:* The benefit of MARS is obtained when parent lines have complementary traits and phenotyping is accurate enough for QTL mapping using one year of data. In this study, Sureño contributed only four minor grain mold QTL and heritability was low.
- *Project adjustment:* MARS was not deployed because the QTL effect would not sufficient to accumulate grain mold resistance in the Nganda background.
- *Lesson learned:* While MARS may be effective to accumulate multiple traits with simple genetic architecture, it is less likely to succeed for complex traits with low heritability (such as yield and grain mold resistance). In this case, it will be more effective to use immortalized mapping populations that have been designed to control for phenotypic covariates that confound phenotyping.

C. Student training achievements

To build the foundation for a genomics-enabled breeding network, six West African PhD students were trained (in U.S and West Africa) for scientific leadership roles; and three students were trained for scientific support roles (MSc and License) in West Africa.

US-based PhD and leadership training

PhD trainee geneticists at KSU (Fanna Maina and Jacques Faye) developed expertise in (i) population and quantitative genomics, (ii) bioinformatics and computations (R, Linux, Python, and Java), (iii) and knowledge of allied disciplines including field breeding, stress physiology, and molecular biology. Maina received training on lysimeter drought phenotyping at ICRISAT-Niger. Both trainees developed English proficiency and communication skills e.g. papers for international journals and presented posters at several national and international conferences.

WA-based graduate and leadership training

PhD trainee breeders at CERAAS (Cyril Diatta, ISRA; Eyanawa Akata, ITRA) developed expertise in (i) field breeding, (ii) crop stress phenotyping, (iii) genetic/genomic analysis. Diatta conducted a PRA for Senegal (four regions) that led to a major revision of ISRA breeding targets (*Striga* resistance, open panicle varieties, etc.), while Akata designed a PRA for Togo (pending funding). WACCI PhD trainee Ardaly Ousseni has leveraged SMIL-funded *Striga* phenotyping capacity for an integrated *Striga* management doctoral project and conducted a PRA (two regions in Niger) to guide future INRAN breeding efforts. PhD trainees presented posters at the international sorghum conference in Cape Town.

Institute	Name	Surname	Degree	Status	Gender	Country	Support	Student-led publications
KSU	Fanna	MAINA	PhD (KSU)	Expected 2019	Female	Niger	Full	Maina et al. 2018 Genome
KSU	Jacques	FAYE	PhD (KSU)	Expected 2020	Male	Senegal	Full	Faye et al. in review Mol Ecol
CERAAS	Cyril	DIATTA	PhD (WACCI)	Completed	Male	Senegal	Full	
CERAAS	Eyanawa	AKATA	PhD (UCAD)	Completed	Male	Togo	Full	Akata et al. 2018 Afr Crop Sci J
CERAAS	Nadré	GBEDIE	MS (UCAD)	Completed	Male	Côte d'Ivoire	Full	
CERAAS	Aïcha	GUEYE	Licence	Completed	Female	Senegal	Full	
CERAAS	Awa	NDIAYE	MSc (ENSA)	Completed	Female	Senegal	Full	
KSU	Marcus	OLATOYE	PhD (KSU)	Completed	Male	Nigeria	Data	Olatoye et al. 2018 G3
INRAN	Ardaly	OUSSENI	PhD (WACCI)	Expected 2019	Male	Niger	Data	

D. Short-term training and outreach

Training	Timing	Location	Aim	Participants	Gender
Striga phenotyping	4 weeks (2015)	ICRISAT-Mali	Build INRAN's field *Striga* phenotyping capacity, covering *Striga* collection, experimental design, and electronic data management	Ardaly Ousseni	1M
Genomics and bioinformatics workshop	1 day (2016)	Saly, Senegal	Introduction to genomic analysis and bioinformatics on the open source R platform (Morris with SMIL PIs Tuinstra and Weil)	CERAAS and INRAN students	2F+12M
Seminars on genomics-enabled breeding	2 hours (2015-2018)	CERAAS/ Virtual	Introduce genomics concepts. Morris: "Improving sorghum adaptation with genomics-enabled breeding". Rami: "Use of molecular marker in sorghum breeding". Faye: "Population genomics of sorghums landraces of Senegal"	Students from CERAAS, ENSA, and KSU, CERAAS staff	8F+10M 10(F+M) 9F+6M
Genetics and communication training	2 month (2017)	KSU	Training on genetic analysis, share phenotyping for drought tolerance findings with KSU trainees, and prepare collaborative publications	Eyanawa Akata, CERAAS PhD trainee and ITRA-Togo Sorghum Breeder	1M
Lysimetric drought phenotyping	2 month (2017)	ICRISAT-Niger	Build CERAAS's drought phenotyping capacity by training the technician who runs the lysimetic system at CNRA Bambey (Falalou Hamidou)	Romiel Badgi, CERAAS field technician	1M
Seed multiplication techniques	1 day, various (2017)	INRAN	Strengthen the capacity of producers in sorghum seed multiplication techniques (Aissata Mamadou & Magagi Abdou)	30 farmers and 10 technicians	10F+M49

6 - UTILIZATION OF RESEARCH OUTPUTS

A. Technologies and management practices by phase of development

- Biological: Sorghum varieties with improved adaptation traits
  - Phase of development: I (under research)
- Biological: Genetic markers for adaptation traits in sorghum
  - Phase of development: I (under research)
- Management Practice: A genomics-enabled plant breeding network that facilitates scientific discovery and accelerates sorghum plant breeding
  - Phase of development: I (under research)

B. Intermediate outputs

- Reference germplasm set (WASAP) for West African sorghum programs (seeds increased and available for distribution by CERAAS)
- Reference SNP genotyping (marker) data sets for sorghum germplasm from Senegal, Niger, Togo, Mali, Gambia, Mauritania, and Nigeria (available on NCBI-SRA and Dryad).
- Reference agro-morphological phenotyping data sets for sorghum germplasm from Senegal, Niger, Togo, and Mali.
- New source of drought tolerance identified in the West African germplasm.
- New candidate *Striga* resistant lines developed in Niger.
- Candidate markers for water-limited yield, *Striga* resistance (LGS1 locus), and local adaptation.

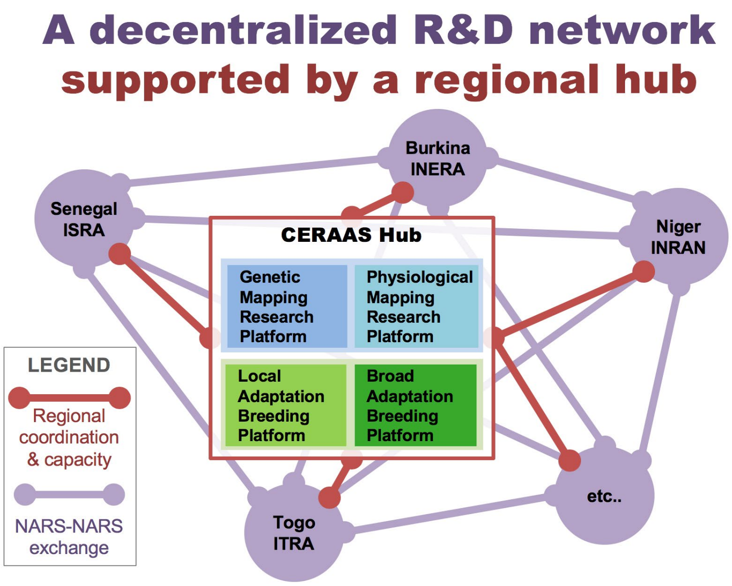

FIG. 2: **In phase-I we built a *decentralized R&D network* which we would formalize and reinforce in phase-II.** We believe this network is the best system to deliver smallholder-responsive varieties in the near-term and long-term. This structure is thought generate the greatest gains in breeding programs, by maintaining breeder autonomy while providing greater resources and positive accountability (Cooper et al. 2014). CERAAS, as regional center, is the natural hub for this R&D network. NARS-NARS exchange of germplasm, data, and technology will be facilitated by the hub. The hub will also leverage its unique scientific capacity to develop knowledge and tools for the NARS and provide training.

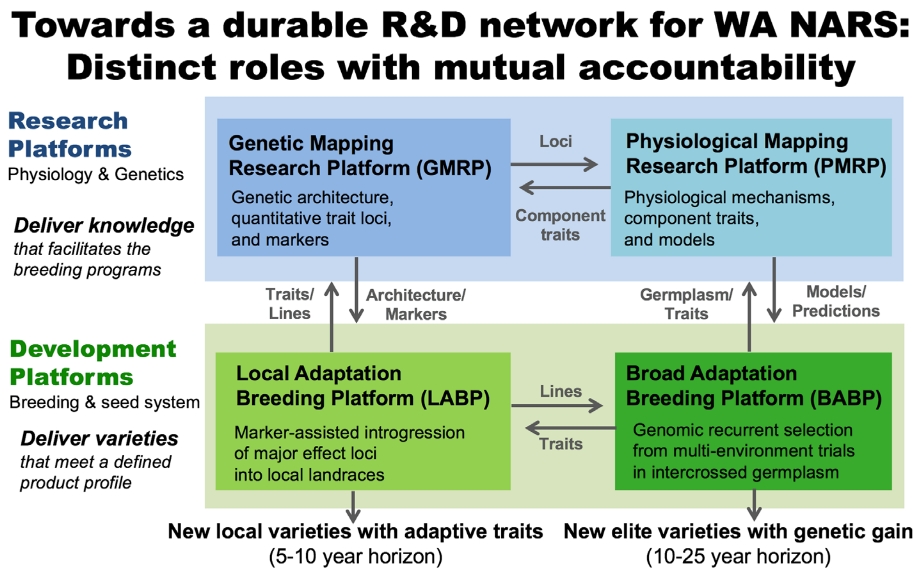

FIG. 3: Research and development (R&D) platforms in the CERAAS hub have distinct roles with mutual accountability. Specialization of roles improves the efficiency of each component, while exchange of intermediate outputs (gray arrows) provides mutual accountability across the system; from research, to development, to delivery of new varieties to smallholder farmers.

7 - STATEMENT ON PROPOSED FUTURE ACTIVITIES FOLLOWING PHASE I RESEARCH ACCOMPLISHMENTS

A. Statement on future activities in phase II

Phase-II Goals:
Our central Phase-II activity will be to **implement genomics-enabled breeding** for development of **stress-adapted, stakeholder-responsive sorghum varieties** for smallholder farmers in West Africa. These two overall goals are to be achieved by the end of Phase-II (2023):

Goal 1: Develop new stress-adapted sorghum varieties that respond to stakeholder needs (G1)

- The genomics-enabled breeding activities will be carried out to develop new candidate varieties.
- Seed system collaborators will help develop criteria for selection and identify varieties for release.

Goal 2: Establish a durable sorghum R&D network for West African NARS (G2)

- Build an R&D network that employs best practices for (i) scientific rigor and (ii) stakeholder responsiveness (based on comparison to global NARS, feedback from a scientific advisory board).
- Move towards international recognition of CERAAS as the regional hub for a high-performing West African NARS R&D network.

Phase-II Research & Development Platforms:

Network organization and governance

- In phase-II, the capacity developed in phase-I will be **deployed in an applied genomics-enabled breeding (GEB) program** with four platforms, described below (Fig. 2-3).

- Each of the four platforms will be **led by an early-career scientist (including SMIL trainees)**. Each early-career scientist will be mentored in this new leadership role by an established colleague.

- The project will support a **decentralized R&D network** (Cooper et al. 2014) of West African NARS breeders. The network is *decentralized* because decisions will made by individuals NARS breeders, and the hub will coordinate and build capacity based on NARS input (Fig. 2).

- **Maintaining and enhancing decision-making at the NARS** level is necessary for durability of the network. We believe a centralized, top-down organization would not outlive the project.

- Breeding activities will be defined and managed as a *product development* not as a *research*. This approach, advocated by Gary Atlin (BMGF, formerly CIMMYT; Atlin et al. 2017), maintains the **breeding programs focus on delivery of varieties, with accountability to smallholders** (Fig. 3).

- With defined product profiles and breeding targets established, research activities will be prioritized by the breeding programs. The **research programs will be accountable to deliver knowledge and tools that facilitate the breeding program** (Fig. 3).

- The platforms will be run out a **CERAAS Hub**, which will be primarily responsible for coordinating exchange (e.g. germplasm, data, technology) and building scientific capacity in the network.

- **CIRAD, ICRISAT, and KSU collaborators will support implementation, training, and outreach**. CIRAD will be primarily responsible for supporting the Development Platforms (variety delivery) and KSU will be primarily responsible for supporting the Research Platforms (knowledge delivery).

Local Adaptation Breeding Platform (LABP)

- This platform will be used to develop new versions of **locally-adapted, locally-preferred varieties that carry new stress-tolerance traits**.

- This platform will be focused on **near-term delivery of varieties (5-10 year horizon)** taking advantage of **established genetics** (e.g. stay-green drought tolerance and *Striga* resistance loci).
- Participatory Rural Appraisal and seed system input will be used to prioritize targets (local varieties + stress-tolerance traits) for the platform (Weltzien et al. 2008).

- This platform will use **rapid-cycling marker-assisted backcross (MABC)** in nurseries and greenhouses to transfer a limited number of genomic variants from donor lines into local varieties.

- **Participatory breeding activities with seed system partners** will be used to test the hypothesis that local-preference traits have been retained.

- **Managed stress trials (MSTs) and multi-environment trials (METs)** will test the hypothesis that local adaptation has been maintained and new stress-tolerant traits have been obtained.

- This platform seeks to build off success of local adaptation programs of ICRISAT (2000s; e.g. *Striga*-resistant Hugurtay in Eritrea; Yohannes et al. 2015), while **using out-sourced genomic technology to accelerate trait transfer** and recovery of the local genetic background.

- The LABP will be led by Jacques Faye (KSU/CERAAS). The mentor will be Jean-Francois Rami (CIRAD).

Broad Adaptation Breeding Platform (BABP)

- This platform will be used to develop a **broadly-adapted germplasm** for varietal development in WA.

- This program will be focused on **long-term genetic gain and adaptability (10-25 year horizon)** through the development of **novel genetics**.

- To **facilitate genetic exchange among NARS**, we will use nuclear male sterility, transferred from SMIL-Haiti project. Increased diversity and recombination is expected to increase genetic gain.

- The novel germplasm and performance data (MET and MST) developed through this platform will form the basis of a **regional genomic selection program** to launch in the later years of phase-II.

- **Participatory breeding and collaboration evaluation** of grain quality will be used to ensure that varieties developed **conform to local preferences, improving likelihood of adoption**.

- This platform seeks to build off success of broad adaptation programs of IRAT (1960-1970s; e.g. IRAT204) and ICRISAT (1970-1980s; e.g. SEPON82), while using collaboration to address shortcomings that limited varietal adoption from these earlier programs (Walker & Alwang 2015) and **genomic technology to improve genetic gain**.

- The BABP will be led by Cyril Diatta (ISRA). The mentor will be Aissata Mamadou (INRAN).

Genetic Mapping Research Platform (GMRP)

- This platform will (i) generate **genetics knowledge that guides breeding** and (ii) develop **genetic tools to facilitate breeding** (trait-predictive or background markers, genomic predictions).

- This platform will generate knowledge on the genetic architecture (gene number, gene effects sizes, gene action, linkage) of adaptive traits, which is needed to **design effective breeding strategies**

- This platform will generate publications that inform the global crop genetics community and build the scientific reputation of the CERAAS hub and West African NARS.

- This platform will generate **trait-predictive markers** for the LABP and BABP, using data from the PMRP (see below). These markers will also facilitate the PMRP by accounting for known genes.

- This platform will also use **genomic data sets to generate markers for local genetic backgrounds** to facilitate recovery of local genetic background in the LABP (Yohannes et al. 2015).

- The GMRP will be led by Fanna Maina (KSU/INRAN). The mentor will be Abdoul-Aziz Saidou (CIRAD).

Physiological Mapping Research Platform (PMRP)

- This platform will (ii) **generate physiology knowledge that guides breeding** and (ii) **develop physiological tools to facilitate breeding** (traits, phenotyping methods, reference checks).

- This platform will generate **knowledge on the physiological mechanisms of adaptive traits**, which is needed to design effective phenotyping strategies for breeding and genetics.

- This platform will **develop novel traits and phenotyping tools for genetics and breeding** programs.

- Modeling will be used the target lines to test in each targeted environment, to help use resources most effectively. We have recruited CERAAS biometrician/modeler Diarietou Sambakhe (PhD, 2018) as a collaborator.

- This platform will generate publications that inform the global crop science community on stress adaptation and build the reputation of West African NARS.

- The PMRP will be led by Bassirou Sine (CERAAS). The mentor will be Falalou Hamidou (ICRISAT).

Phase-II Specific objectives, hypotheses, and anticipated outcomes:

For future activities, we developed specific objectives via our goal-directed, hypothesis-driven R&D framework. The specific hypotheses (i) facilitate crop improvement activities in the near-term through scientific rigor and accountability and (ii) facilitate crop improvement in the long-term through better understanding of physiological and genetic mechanisms of abiotic and biotic stress adaptation. We provide some examples specific objectives here:

Objective: Develop locally-preferred varieties with stay-green post-flowering drought tolerance in 5 years

- *Hypothesis:* The *stg1-4* stay-green alleles from B35 will confer post-flowering drought tolerance to WA local varieties (Borrell et al. 2014; Ouedraogo et al. 2017).

- *Activities:* Conduct MABC of *stg1-4* into locally-preferred varieties (based on PRA) from Senegal, Burkina Faso, Togo, and Niger using the LABP. Test for improved post-flowering drought response under managed drought stress (CNRA-Bambey) and in farmer fields, compared to original varieties.

- *Anticipated outcome:* New drought tolerant versions of local varieties for release in Senegal, Togo, Burkina Faso, and Niger. (G1)

Objective: Develop locally-preferred varieties with low germination-stimulant Striga resistance in 5 years

- *Hypothesis:* The $lgs1_{SRN39}$ *Striga* resistance allele from SRN39 will confer *Striga* resistance to WA local varieties (Gobena et al. 2017).

- *Activities:* Conduct MABC of $lgs1_{SRN39}$ into locally-preferred varieties (based on PRA) from Senegal, Togo, Burkina Faso, and Niger using the LABP. Test for reduced *Striga* infestation under managed *Striga* stress (INRAN-Konni) and in farmer fields, compared to original varieties.

- *Anticipated outcome:* New *Striga*-resistant versions of local varieties for release in Senegal, Togo, Burkina Faso, and Niger. (G1)

Objective: Develop breeding product profiles that accurately reflect smallholder demand

- *Hypothesis:* Product profiles provide detailed information on consumer's preferences that can be linked to the ideotype of new varieties.

- *Activities:* Conduct Participatory Rural Appraisals and surveys/interviews with farmer organizations in Burkina, Senegal, Niger, and Togo. Breeder workshops to refine and prioritize product profiles.

- *Anticipated outcome:* Reliable product profiles that guide breeding programs to smallholder responsive varieties and engage stakeholders (local partners, funders, collaborators) [G1, G2]

Objective: Integrate farmers organizations in a participatory breeding system to enhance adoption

- *Hypothesis:* The tolerant/resistant lines with novel *Stg/lgs1* alleles grown in farmers' field will facilitate their adoption by farmers in WA with consistent performance across environments.

- *Activities:* Implication of farmer in variety development and participatory evaluation of tolerant/resistant lines and locally adapted varieties in farmer's field and research stations.

- *Anticipated outcome:* Identification of locally-preferred varieties adapted to farmers' conditions and favorable for adoption.

Objective: Develop novel drought avoidance traits via root system architecture design

- *Hypothesis:* Genotypes with "T"-shaped root system architecture (some roots very shallow, some very deep) will avoid pre- and post-flowering drought compared to those with "Λ"-shaped root systems.

- *Activities:* Mapping root traits in biparental populations (deep X shallow roots, now at F7) and assess the role of root architecture in drought avoidance in managed stress field and lysimeter experiments.

- *Anticipated outcome:* Design suitable drought tolerant ideotypes matched to agroclimatic zones. [G2]

Objective: Manage photoperiodic flowering along climatic variation in WA.

- Hypothesis: Introgression of maturity genes (e.g. *Ma1-6*) will enhance sorghum adaptation to specific environments in WA.

- Activities: Introgress maturity genes into photoperiod sensitive landraces; determine effect of planting dates on flowering time across climatic zones in WA.

- *Anticipated outcome:* New photoperiod insensitive and early maturing varieties combined with desired agro morphological traits.

B. Linkage to Phase I objectives and activities

Our phase-II activities will be direct extensions of phase-I activities:

- Linkage type-1: Deploy the genomic-enabled breeding resources from phase-I for ***varietal development in phase-II***.

- Linkage type-2: Address gaps in our phase-I activities to improve likelihood for the network's ***impact and durability beyond phase-II***.

We provide some examples in the table below.

Type	Area	Need	Phase-I activity	Phase-II activity	Deliverable
1	Breeding	Phenotyping capacity for managed stress for NARS breeding programs	Developed *Striga* and drought phenotyping capacity at INRAN, trained NARS techs in phenotyping	Maintain/rebuild *striga* and drought capacity at INRAN, build targeted phenotyping capacity with other NARS	Reliable phenotype data to guide varietal development [G1]
1	Breeding	NARS need better info on traits required to drive adoption of new varieties	Conducted Participatory Rural Appraisals, developed product profiles by breeding programs,	Develop and implement a full participatory breeding framework, integrated in the genomics-enabled workflow	Varieties that are acceptable, with at least one target trait to drive adoption [G1]
1	Research	Breeding programs need novel drought tolerance traits	Phenotyped of diverse WA germplasm under managed drought stress, mapping for drought tolerance markers	Confirm and characterize candidate drought tolerance sources in multi-environment and managed stress trials	2-5 novel drought tolerance donors with trait-predictive markers [G2]
2	Training	SMIL PhDs need highly-qualified staff in order to implement their GEB programs	Short-term training for technicians on phenotyping, genetics, and breeding management software	Long-term training (in-country Masters degrees) for technicians at CERAAS hub	MSc-level technicians at CERAAS hub for physiology, breeding, & genetics [G1]
2	Training	SMIL PhDs and early-career collaborators need postdoctoral training to implement their programs	Long-term PhD training in US and WA to develop genomics, genetics, breeding, and physiology knowledge	Extended short-term training (4-6 month) for early-career investigators, with visiting scientist appointments at KSU	Publications in international journals, strengthened network & communication [G2]

C. Training and outreach objectives

The training and outreach activities are aimed at (i) helping the PhD trainees successfully implement their programs and (ii) improved the long-term durability of the R&D network.

Training

Masters training for hub technicians (CERAAS genetics, CERAAS physiology, and ISRA breeding)

- *Aims:* Improve the quality/capacity of foundational research at CERAAS (G2) and advance professional training of women scientists [G2]
- *Details:* Masters training in Senegal for technicians; short-term training experiences at CIRAD

Leadership and management training for PIs and staff scientists

- *Aims:* Provide early- to mid-career scientists leadership and managements skills needed to navigate complex, trans-disciplinary R&D projects in multi-cultural teams [G2]
- *Details:* Extended short-term Short-term training be conducted by hfp consulting, scientific leadership training providers used by BMGF

Graduate training modules at West African Center for Crop Improvement (University of Ghana)

- *Aim:* Leverage PhD training of KSU students by having them provide instruction at WACCI, with PIs
- *Details:* Modules would be focused on genetics, genomics, and application of the scientific method

Outreach

Seed system integration for project activities via outreach to farmer organizations and seed companies

- This would be conducted in partnership RESOPP farmer cooperative network in Senegal, the LSDS-Halal seed company in Niger, and other seed system stakeholders. [G1]
- Based on consultation with the seed systems, we would develop and implement a participatory breeding approach of mutual benefit. [G1, G2]

Interactions with global scientific community to establish international reputation of NARS

- Build CERAAS as a recognized regional scientific hub and West African NARS scientists as full members of global scientific community.
- Regular attendance at international meetings (i.e. including those in North America and Europe).
- Visiting scientist opportunities in U.S. for early-career NARS collaborators to strengthen professional relationship and communication skills. [G2]
- Regular publication in international scientific society journals, including targeting a small number of flagship publications to selective journals. [G2]

8 - LITERATURE CITED

*(SMIL Trainees in **bold**.)*

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SORGHUM TRAIT DEVELOPMENT PIPELINE FOR IMPROVED FOOD AND FEED VALUE

1 – PRINCIPAL INVESTIGATOR

Mitch Tuinstra - *Purdue University, USA*

2 – RESEARCH TEAM

Co-Investigators: Clifford Weil – *Purdue University, USA*
Brian Dilkes - *Purdue University, USA*

Partners: Soumana Souley – *INRAN, Niger*
Aissata Mamadou – *INRAN, Niger*
Ndiaga Cisse - *CERAAS/ISRA/CNRA, Senegal*
Khalil Kane - *ISRA/LNRPV, Senegal*

3 - PROJECT GOALS AND OBJECTIVES

Sorghum is poised to play a key role in expanding agricultural development and food security in countries around the world. New marketing opportunities for sorghum include use in high-quality food and beverage products and feed for livestock production. Success in meeting these demands hinges, in part, on the successful transfer of genetic, genomic, and agricultural technologies that have been developed for the crop.

The goal of this project is to leverage existing genetic technologies to develop novel traits that enhance the value and performance of sorghum. We are using the sorghum genome sequence, a population of nearly 600 sequence-indexed mutants, a population of more than 11,000 random mutants, and a collection of 847 diverse sorghum accessions from around the world to identify and characterize allelic variation in genes that influence grain and forage quality traits. In consultation with our collaborators in West Africa, we are executing projects to: 1) improve protein digestibility, 2) reduce phytate content, 3) develop modified starch composition traits, 4) create starches with altered gelatinization temperature, and 5) improve forage quality for livestock feed. These studies are contributing to a better understanding of the genetic and biochemical bases for these new traits in sorghum.

We are addressing capacity development goals through short and long term training and education programs. We are training national program plant breeders and students in the use of genetic, genomic, and phenomic data and the tools for crop improvement. We are making these data and genetic resources available in their home countries.

The crop development activities are producing new and unique sorghum genotypes, varieties, and hybrids with enhanced food- and feed-quality traits that can be grown in Niger and Senegal. In the medium- to long-term, farmer participation in evaluation and selection of these varieties will promote acceptance and production of new cultivars. The existing linkages with the private seed industry will contribute to rapid commercialization of new cultivars. Increased production of high-quality grains will stimulate and support development of new markets.

4 - OVERVIEW OF ACTIVITIES

Tremendous gaps remain in our understanding of the valuable traits contained in sorghum. This project is developing the genetic resources to improve key quality traits of sorghum grain and forage products.

Improving Protein Digestibility of Sorghum after Cooking

Sorghum has lower protein digestibility compared to other cereals such as maize, wheat and rice. Sorghum protein digestibility decreases to less than 50% when it is wet-cooked. In previous studies, researchers at Purdue University showed that the sorghum mutant, P721Q, exhibited increased protein digestibility compared to normal sorghums. Unfortunately, P721Q and derived lines exhibited floury endosperm and soft seeds that limited the value of this mutant for crop improvement.

In this project, Elisabeth Diatta, a Ph.D. student from Senegal, renewed efforts to identify and characterize genetic variants with improved protein digestibility. Early analyses of protein digestibility focused on the standing genetic variation of sorghum in the West Africa Sorghum Association Panel (WASAP). Protein content of the WASAP accessions was measured using Near Infrared Spectroscopy (NIRS) and showed that protein content ranged from 7 to 18 g/100 g of samples. Samples from Niger were found to have the largest variability for protein content (7 to 18 g), followed by Senegal (10 to 17 g), then Mali (10 to 16 g), and Togo had the least variability for protein (12 to 16 g). Analyses of protein digestibility showed that these accessions ranged from 0 to 55% protein digestibility after wet cooking. Protein digestibility and Total Nutrient Digestibility were found to be negatively correlated with Tannin content and Acid Detergent Fiber (Fig. 1). Samples from Niger exhibited the greatest variability in digestibility, followed by Togo, Senegal, and Mali. None of the 385 accessions in the WASAP exhibited highly digestible protein after wet cooking (i.e. with 60% digestibility or more); hence efforts were made to find or create other sources of genetic variability for this trait.

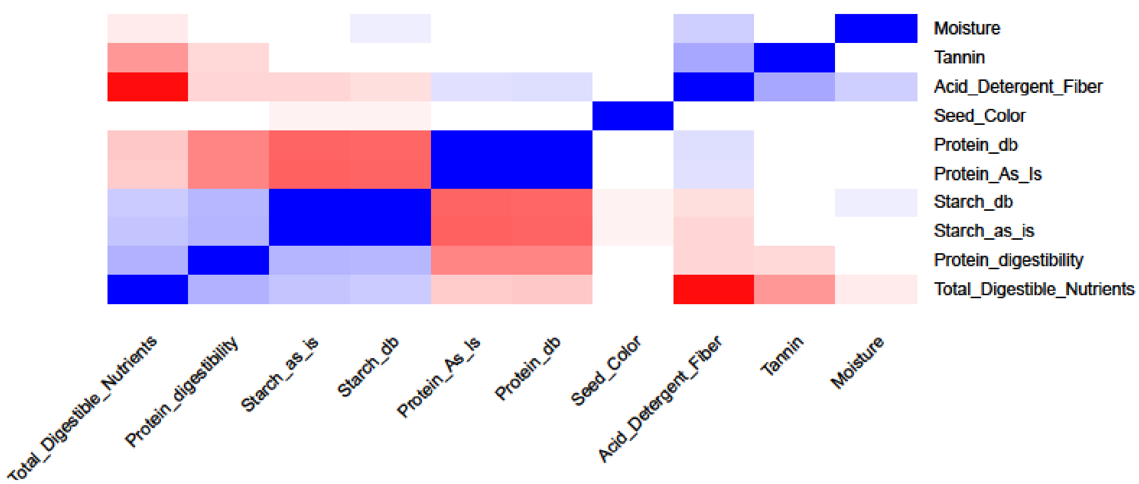
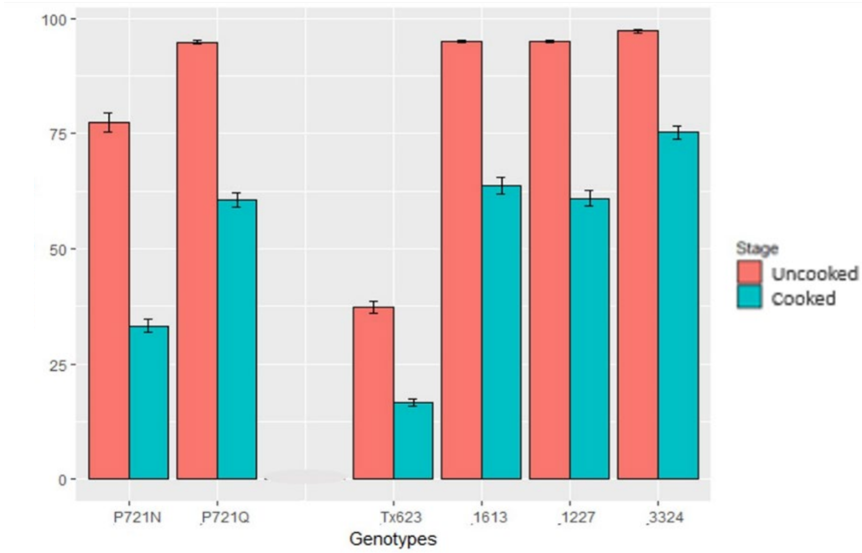


Figure 1. Heat map showing correlations between seed characteristics, protein digestibility, and total digestible nutrients properties of sorghum accessions in the WASAP.

Subsequent analyses of protein digestibility focused on chemical mutants from an ethyl methane sulfonate (EMS) sorghum population. A forward genetic screen identified three EMS mutants with improved protein digestibility (Fig. 2). A bulked segregant analysis (BSA) with genome sequencing revealed that the digestibility phenotype of two of the new mutants mapped to two different loci on chromosome 5. The highly digestible phenotype of SbEMS1613 was found to be linked to a point mutation in the coding sequence of a 26S proteasome subunit. The highly digestible phenotype of SbEMS3324 was controlled by a point mutation in the coding sequence of a α-kafirin gene. These SNPs will enable marker assisted breeding for introgression of the protein digestibility traits into elite sorghum lines.

Figure 2. Comparison between percent protein digestibility of mutant P721Q and its parent line P721N, and between EMS lines 1613, 1227, 3324 and their parent line BTx623 after wet cooking with the standard errors.



Reducing Phytate Content of Sorghum to Improve Iron Bioavailability

The presence of phytic acid in sorghum and other cereal porridges inhibits the uptake of iron by humans; as a result, decreasing phytic acid levels in sorghum grain would improve nutritional quality. There are three genes known to impact phytic acid levels in the seed of maize. BLAST analyses identified five sorghum genes that corresponded to these maize genes. Our sequence-indexed mutant lines included 13 genotypes that have missense mutations in one of the five genes involved in phytate accumulation. We evaluated seeds of these homozygous genotypes for phytate content using a colorimetric test but did not identify significant variation in phytate among genotypes. Given this lack of variation, we ended this research effort.

Identifying New Alleles that Contribute to High Amylose Content in Sorghum Grain

A major step towards an economically viable sorghum grain market beyond subsistence farming is the development of new food and non-food uses for the crop to expand staple food markets. A first step towards this goal is the development of sorghum varieties with modified starch properties such as higher amylose content. In corn, high amylose starches are resistant to digestion by amylases and have unique physical and chemical properties that are valuable for food and industrial uses because of their relatively unbranched structure. We are working to create similar sorghum starches that have added value for new product development.

Few variants in starch composition are known in sorghum. Stefanie Griebel, a Ph.D. student working on this project, adapted an alkali test originally developed to classify starch quality in rice for use in sorghum. Cut sorghum seeds were screened with 1.8% KOH for 24h. Our initial analyses of Alkali Spreading Value (ASV) in elite U.S. sorghum breeding lines indicated little variation. Seed samples from BTx623 and Sepon82 did not show any swelling or disintegration at 1.5% and 1.8% KOH concentrations after 24h or 48h (Fig. 3). The screening program was expanded to evaluate 5,720 sorghum EMS mutants. Ten SbEMS mutants were identified with strong ASV phenotypes similar to observations of ASV in rice (Table 1). These mutants exhibited strong swelling phenotypes suggesting potential changes in starch content, composition, or physical properties. The ASV phenotypes for most of the sorghum genotypes were similar at 1.5% KOH and 1.8% KOH, so it was decided to use 1.8% KOH as the optimal concentration (Fig. 3). Incubation periods of 24h and 48h were evaluated and no significant differences in the appearance of seeds showing ASV+ were observed (Fig. 3).

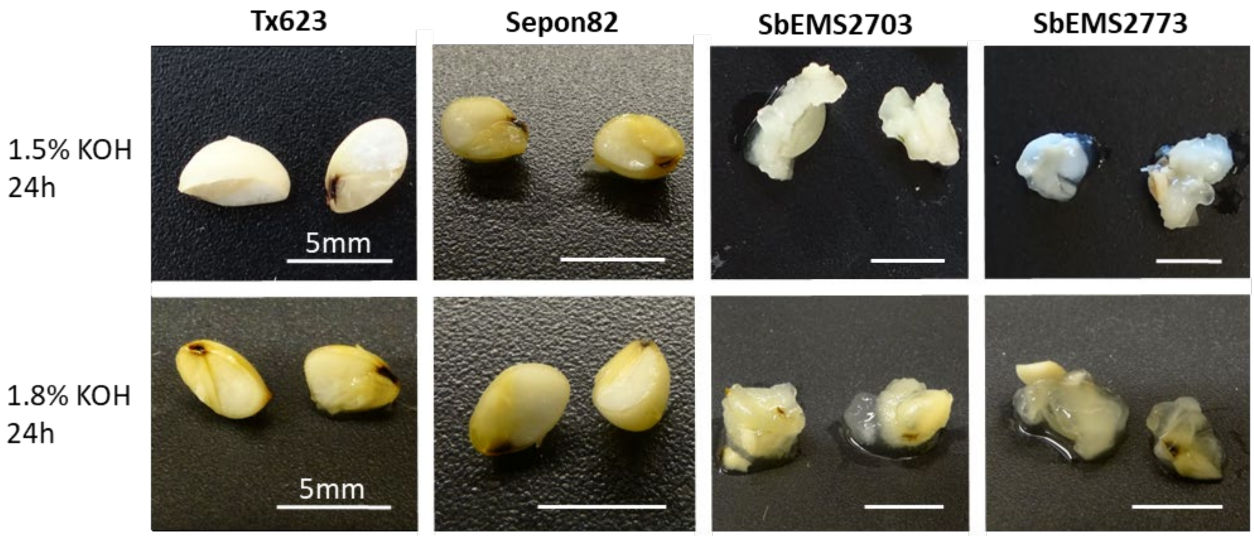


Figure 3. Examples of Sorghum bicolor EMS mutants that exhibit ASV- (Tx623 and Sepon82) and ASV+ (SbEMS2703 and SbEMS2773) after 24h treatment with 1.5%KOH and 1.8%KOH.

EMS mutants with strong ASV+ phenotypes exhibited a range of starch gelatinization temperatures (GT) that ranged from lower to higher than the wild-type Tx623 samples (Table 1). Higher ASV scores were correlated with lower enthalpies and greater starch GT ranges. Whole genome sequencing revealed candidate SNPs in genes related to starch biosynthesis. Each mutant was crossed to BTx623 and segregation analyses showed that mutations in genes for starch synthase and starch branching enzyme co-segregated with the ASV phenotype.

Table 1: Starch gelatinization characteristics (starch:water 1:3 ratio) of sorghum genotypes produced in West Lafayette 2016 and Puerto Rico 2016/2017 and rice checks.

Genotype	Type	Biological reps	N	Starch Gelatinization Characteristics T_o [°C] lsmean		T_p [°C] lsmean		T_c [°C] lsmean		T_c-T_o [°C] lsmean		Enthalpy [J/g]		[1]Melting Point [°C]	
SbEMS 2703	EMS mutant	6	12	68.3	ab	78.9	a	90.3	ab	22.0	ab	3.0	cd	98.2	a
SbEMS 2773	EMS mutant	6	12	68.5	a	78.7	a	90.9	a	22.5	a	4.2	abc	98.0	a
SbEMS 3194	EMS mutant	6	12	66.2	c	77.0	bc	87.1	cde	20.8	ab	2.0	d	97.3	a
SbEMS 3218	EMS mutant	6	12	67.8	abc	78.0	ab	88.3	bcd	20.4	abc	2.4	d	98.2	a
SbEMS 3403	EMS mutant	5	10	66.5	abc	76.3	cd	88.6	abcd	21.9	ab	3.3	bcd	98.4	a
SbEMS 3568	EMS mutant	4	8	66.3	bc	77.1	bc	87.6	cde	21.4	ab	2.1	d	98.0	a
SbEMS 3920	EMS mutant	5	10	58.7	d	66.7	f	78.9	f	20.0	abc	3.4	bcd	96.2	a
SbEMS 4308	EMS mutant	5	10	56.0	e	63.5	g	76.1	g	20.2	abc	3.1	cd	96.0	a
SbEMS 4565	EMS mutant	5	10	68.8	a	77.2	bc	88.4	bcd	19.6	bc	3.5	bcd	97.0	a
SbEMS 5890	EMS mutant	6	12	68.4	ab	78.7	a	89.2	abc	20.8	ab	2.2	d	98.1	a
Sepon82	Check	2	5	67.2	abc	72.7	e	84.6	e	17.4	cd	5.4	ab	98.0	a
Tx623	Check	2	6	69.7	a	73.7	de	85.2	de	15.5	d	5.8	a	97.6	a
Century Patna 231	Rice check	1	3	73.6	A	78.9	A	89.2	A	19.3	A	6.4	A	100.4	A
Magnolia	Rice check	1	4	60.8	B	69.3	B	80.1	B	15.6	B	2.8	B	97.7	A

T_o= onset starch GT, T_p= peak starch GT, T_c= conclusion starch GT

Values followed by the same letters in the same column are not significantly different ($p<0.05$).

[1]Tx623, Sepon82 N=4; SbEMS2773 N=11; SbEMS4565, SbEMS4308 N=9; SbEMS3403 N=7; SbEMS3920 N=8; Century Patna 231 N=1.

Analyses of genotypes with mutations in starch branching enzyme showed that these genotypes were characterized with higher starch GT, higher amylose values and lower viscosity than wild type starches. These alleles contributing to novel starch traits are being introgressed into elite sorghum varieties for crop improvement. Sorghum starches with unique quality attributes may play a role in new food and beverage products.

New alleles for starches with altered gelatinization temperatures will be identified

Our initial efforts to discover genes and alleles for starches with modified GT focused on analyses of the standing genetic variation in the Sorghum Conversion Program. The ASV test was used to screen grain samples of approximately 850 sorghum conversion lines. Less than 5% of the SC-lines exhibited weak ASV+ phenotypes. None of these variants produced ASV+ phenotypes as strong as the EMS mutants described in Table 1. In ongoing studies, 4 donor SC-lines with weak ASV+ phenotypes were crossed with Tx623 to create bi-parental mapping populations to map and discover the genes involved in these ASV+ traits.

Some of the EMS mutants with ASV+ phenotypes shown in Table 1 have mutations in a starch synthase. Starch quality analyses demonstrated that these mutants exhibit lower starch GT, normal amylose content, and slightly lower viscosity than starches from wild type plants. These alleles contributing to lower starch GT have been crossed to elite food-grade sorghum varieties from West Africa (see Objective 2 below) to create breeding populations for crop improvement. The development of varieties with lower starch GT may play a role in development of new food and beverage products.

New alleles discovered for improved forage quality

Sorghum stover was shown to be among the most important feed options for livestock in Niger based on a participatory rural appraisal (PRA) conducted by Ousmane Seyni, a Ph.D. student from NIGER working on the project (Fig. 4). Purdue is working with INRAN to develop new sorghum cultivars with improved forage quality. One component of this work involved efforts to discover new genes and alleles for the brown midrib trait. Purdue University sent seeds of the sequence indexed EMS sorghum population to NIGER. INRAN scientists conducted a forward genetic screen in the population and identified 11 mutants with brown midrib (bmr) phenotypes in the population. Genetic crosses and whole genome sequencing revealed candidate SNPs in genes related to lignin biosynthesis. Genetic complementation studies were used to verify these results and revealed that these bmr mutants represent new alleles of the known genes bmr2, bmr6, and bmr12.

Figure 4. Bundles of sorghum stover in Konni, Niger (courtesy of Ousmane Seyni).

Objective 2. Develop locally adapted sorghum varieties and hybrids having improved grain quality and feed value

Improving protein digestibility

Purdue PIs are working with the sorghum breeding program at CERAAS to initiate breeding programs for sorghum with highly digestible protein. As a first step in establishing the program, Dr. Khalil Kane was invited to Purdue University for short term training on standardized methods for measuring protein digestibility in sorghum. The equipment required for establishing a protein digestibility lab in Senegal were then acquired and sent to the CERAAS research station in Theis, Senegal. Dr. Kane worked with CERAAS staff to establish this lab and support local breeding efforts for this key trait.

Crosses were made between four new open-pollinated lines from ISRA (ISRA-S-621A, ISRA-S-621B, ISRA-S-622A, and ISRA-S-622B) and EMS mutants with the highly digestible protein trait. These populations were sent to Senegal and are being managed by Elisabeth Diatta as a component of her Ph.D. dissertation research project at the West African Center for Crop Improvement (WACCI). Additional crosses were made between the highly digestible sorghum mutants and other food-grade sorghum varieties adapted for production in West Africa (CE-151-262-A1, Macia, TxARG1, and MR732). These populations are being managed at Purdue university. To date, nearly 40 genotypes that combine high protein digestibility and food-grade sorghum grain characteristics have been advanced to the F4 generation. Many of these lines have excellent agronomic and grain quality characteristics and will provide genetic material for future studies that test the functionality and nutritional properties of grain having the highly digestible sorghum traits.

Modified starch composition and GT
Purdue PIs are developing sorghum breeding populations to develop new varieties with enhanced starch quality. EMS mutants with modified amylose content or reduced starch GT (see Table 1) were crossed with food-grade sorghums from West Africa including Sepon82, CE-151-262-A1, Wassa, Macia, MR732, Tx623, and N223. More than 1200 F2 plants from these crosses were self-pollinated and the F2 progeny were evaluated to identify those families that were homozygous for the ASV+ phenotype. A set of 207 homozygous F3 families were advanced to the F4 generation in 2018. Many of these lines have excellent agronomic and grain quality characteristics and will provide genetic material for future studies that test the impact of these designer starches on sorghum flour and food products. These lines also represent an excellent base of genetic material for the breeding program.

Dual-purpose and dedicated forage sorghum
To increase sorghum-based feed availability and quality in Niger, Ousmane Seyni crossed and backcrossed bmr6 and bmr12 into well-adapted Nigerien cultivars for variety development. Analyses of the derived progeny showed variability in grain and dry matter yields in near-isogenic brown midrib versions of El Mota, a farmer landrace, and Sepon82, an improved sorghum variety. This indicated the importance of selecting for genetic background in stover quality improvement. Promising families are being advanced in the breeding program based on stover quality. The best progeny will undergo further backcrossing to create dual purpose sorghum varieties.

Tx631 bmr6 and Tx631 bmr12 were crossed to N223, the seed parent used in the Nigerien hybrid F1-223. A set of 150 F4 lines from these crosses that expressed tan-plant and bmr characteristics were sent to Soumana Souley at INRAN in NIGER. Genotypes with superior performance were crossed and backcrossed to AN223. Single plant crosses between cytoplasmic-genic male sterile line AN223 and bmr lines from Purdue University were planted side-by-side during the 2017 rainy season at Tillabery Research Station. The BC1 and BC2 A-line conversions are being advanced in the INRAN program as potential seed parents of future forage hybrids.

5 - ACCOMPLISHMENTS

A. Achievements by project objectives

Objective 1. Identify genes and alleles that can be used to improve grain quality and feed value of sorghum

1.1 New alleles that enhance protein digestibility will be identified and characterized

- Sorghum has lower protein digestibility compared to other cereals. During the course of this project, 3 EMS mutants were discovered that substantially enhance protein digestibility after wet cooking. These mutants have the potential to transform the nutritional value of this important staple crop.

1.2. New alleles that reduce phytic acid and improve iron bioavailability in sorghum grain will be identified and characterized for impact on end-use processing and nutritional properties.

- The presence of phytic acid in sorghum and other cereal porridges inhibits the uptake of iron by humans. Thirteen mutants were discovered in genes predicted to control phytate content but no variation was detected. Given the lack of variation, we ended this research effort.

1.3. New alleles that contribute to high amylose content will be identified and characterized for impact on end-use processing and nutritional properties.

- High amylose starches are resistant to digestion by amylases and have unique physical and chemical properties. To date, we have discovered 8 different mutations in a key gene involved in starch branching that results in starches with higher GT, higher amylose values, and lower viscosity than wild type starches. There are no prior reports of sorghum genotypes with these starch properties.

1.4. New alleles for starches with altered gelatinization temperatures will be identified

- A key component to starch utility is its gelatinization temperature. Two different mutations in a starch synthase gene were discovered and mutants exhibit lower starch GT, normal amylose content, and slightly lower viscosity than starches from wild type plants. There are no prior reports of sorghum genotypes with these properties.

1.5. New alleles discovered for a brown mid-rib forage quality

- Purdue and INRAN researchers have discovered 11 new brown midrib mutants in an EMS sorghum population. Genetic crosses and whole genome sequencing revealed candidate SNPs in genes related to lignin biosynthesis.

Objective 2. Develop locally adapted sorghum varieties and hybrids having improved grain quality and feed value

We have developed new breeding programs to manipulate the new high protein digestibility traits described in Objective 1. Purdue University is collaborating on crop improvement efforts with our partners at ISRA. A trait conversion program has been developed that focuses on integrating the high protein digestibility traits into ISRA-S-621A, ISRA-S-621B, ISRA-S-622A, and ISRA-S-622B. Purdue University is backstopping these activities with efforts to convert these and other elite sorghum varieties from West Africa for increased protein digestibility.

- The Purdue PIs have created a sorghum breeding program to develop new sorghum varieties with novel starch quality traits.

- The Purdue PIs in collaboration with partners at INRAN have created a sorghum breeding program to enhance sorghum stover quality in Niger.

Objective 3: Conduct long-term and short-term training programs to support institutional capacity building.

See *Student Training Achievements* below.

Objective 4: Gender integration

Disaggregated data has been collected on women and men farmers on trait preferences and potential impacts of newly developed varieties. We identified two excellent and highly qualified female candidates for Ph.D. training.

B. Major challenges encountered and resulting project adjustments

None

C. Student training achievements

Long-term training programs were established to support Ousmane Seyni, a Ph.D. student from Niger, and Elisabeth Diatta, a Ph.D. student from Senegal, in collaboration with the West African Center for Crop Improvement (WACCI). Ms. Stefanie Griebel was also recruited to serve as a third Ph.D. student at Purdue University.

D. Short-term training and outreach

We provided numerous short-term training opportunities for researchers from Senegal, Niger, and Ethiopia. We also provided workshops for the plant breeding students at WACCI and Makerere University on use of the sequence-indexed sorghum resources for research and crop improvement (https://www.purdue.edu/sorghumgenomics/) (Fig. 5). Dr. Tuinstra also provided a presentation at the 21st Century Sorghum Conference that highlighted this resource and protocols for requesting seeds of these EMS mutants on the Germplasm Resource Information Network (NPGS-ARS-GRIN).

6 - UTILIZATION OF RESEARCH OUTPUTS

A. Technologies and management practices by phase of development

Phase I Technologies: Twenty-four different genetic technologies have been discovered in our "Sorghum Trait Development Pipeline for Improved Food and Feed Value" project.

- 3 EMS mutants with substantially higher protein digestibility after wet cooking were discovered and characterized. These mutants have the potential to transform the nutritional value of this important staple crop.

- 8 EMS mutants with high amylose starch content have been discovered and characterized. These mutants have SNPs in a key gene that controls starch branching and produce starches with higher GT, higher amylose values, and lower viscosity than wild type starches. There are no prior reports of sorghum genotypes with these starch properties.

- 2 new mutants with reduced starch GT have been identified and characterized. These mutants have SNPs in a starch synthase gene that controls GT. There are no prior reports of sorghum genotypes with these properties.

- 4 germplasm sources for reduced starch GT have been discovered in the sorghum germplasm collections maintained at Purdue University. Genetic characterization is on-going.

- 11 new mutants with brown midrib phenotypes were identified and characterized. These bmr alleles and other alleles are being used in the forage breeding programs at Purdue University and INRAN for cultivar development.

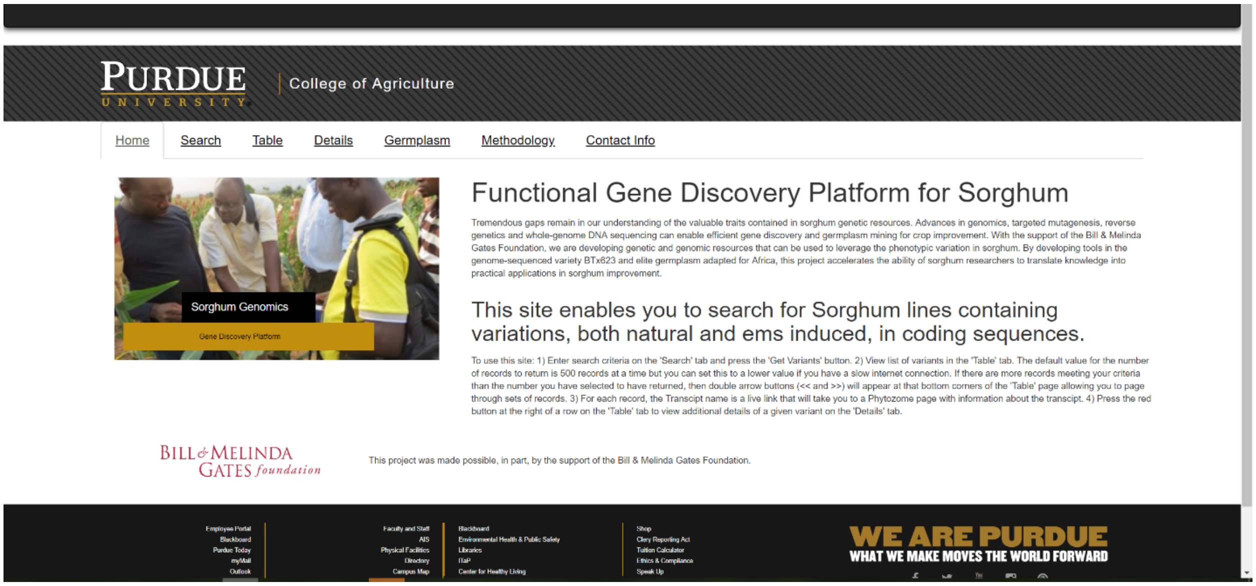


Figure 5. Database for Functional Gene Discovery Platform for Sorghum.

Phase 2 Technologies: We developed new breeding programs to manipulate the high protein digestibility and starch quality grain traits at ISRA and Purdue University. We are also collaborating with INRAN to develop a new sorghum breeding program for enhanced stover quality at INRAN in NIGER.

- A set of 36 selections from the enhanced protein digestibility breeding populations combine high protein digestibility and food-grade sorghum grain characteristics and have been advanced to the F4 generation. Many of these lines have excellent agronomic and grain quality characteristics and represent core genetic material for future studies that test the functionality and nutritional characteristics of new grain sorghum varieties.

- A set of 207 breeding lines that are homozygous for a variety of starch quality traits are being advanced to the F4 generation. Many of these lines have excellent agronomic and grain quality characteristics and will provide excellent genetic material for future studies that test the impact of these designer starches on sorghum flour and food products. These lines represent an excellent base of genetic material for the breeding program.

- Brown midrib versions of El Mota, a farmer landrace, and Sepon82, an improved sorghum variety are being developed at INRAN. Promising families are being analyzed for stover quality and will undergo further backcrossing to create dual purpose sorghum varieties. Brown midrib versions of A/B N223 are being developed at INRAN. The BC1 and BC2 A-line conversions are being advanced in the INRAN program as potential seed parents of future forage hybrids.

B. Intermediate outputs

A protein digestibility research facility was established at CERAAS in Theis, Senegal. Short-term and long-term training were provided for Dr. Khalil Kane and other Senegalese students and scientists for operation of the facility. This program was established to create a center of excellence for sorghum protein research in Senegal.

The forage breeding and livestock research programs at INRAN have established a partnership with Livestock Innovation Lab. Ousmane Seyni, the student supported by this project, has been identified as the future leader of the forage development component of the program after completion of his degree.

7 - STATEMENT ON PROPOSED FUTURE ACTIVITIES FOLLOWING PHASE I RESEARCH ACCOMPLISHMENTS

A. Statement on future activities in phase II

Sorghum is an important, staple food crop poised to play a key role in agricultural development and food security around the world. Until recently, sorghum breeding efforts have mostly focused on increasing grain yield and stress tolerances with little attention given to issues of end-use quality or nutritional value. Consequently, few grain quality traits have been developed in sorghum. However, opportunities in new and expanding markets, especially emerging food and feed markets, will require that more attention be given to developing varieties with preferred quality and nutritional characteristics.

In phase I of this project, the Purdue University "Sorghum Trait Development Pipeline for Improved Food and Feed Value" project developed 24 different genetic technologies that enhance protein digestibility, starch composition, starch gelatinization temperature, or forage quality (Table 2). In many cases, these genes and the alleles identified for crop improvement are the first of their kind developed for sorghum. We have initiated efforts to incorporate these genes into elite varieties through the collaborative breeding programs at Purdue and with collaborators in West Africa. Hundreds of breeding lines are already available as improved trait donors and proof-of-concept varieties for subsequent research and commercialization.

We would like to request a new SMIL project to support research that leverages the genetic technologies and breeding programs developed over the past 5 years to enhance value and performance of sorghum in farmer-accepted varieties adapted in Niger and Senegal (Table 2).

Table 2. Gene donors and associated grain or forage quality traits.

Donor	Trait
SbEMS 1227	High protein digestibility
SbEMS 1613	High protein digestibility
SbEMS 3324	High protein digestibility
SbEMS 2703	High amylose starch
SbEMS 2773	High amylose starch
SbEMS 3194	High amylose starch
SbEMS 3218	High amylose starch
SbEMS 3403	High amylose starch
SbEMS 3568	High amylose starch
SbEMS 4565	High amylose starch
SbEMS 5890	High amylose starch
SbEMS 3920	Low gelatinization starch
SbEMS 4308	Low gelatinization starch
SbEMS 932	Increased forage digestibility
SbEMS 1453	Increased forage digestibility
SbEMS 2354	Increased forage digestibility
SbEMS 2364	Increased forage digestibility
SbEMS 3779	Increased forage digestibility
SbEMS 4465	Increased forage digestibility
SbEMS 4791	Increased forage digestibility
SbEMS 4848	Increased forage digestibility
SbEMS 5095	Increased forage digestibility
SbEMS 5199	Increased forage digestibility
SbEMS 5398	Increased forage digestibility

Our goals in the next phase of the project include:

1. Conduct basic genetic and plant science research as needed to understand the biology of the traits that we have discovered to date. Many of these genes are still poorly understood and more work is needed to learn how to effectively manipulate these traits in sorghum.

2. Expand our collaborative breeding programs with collaborators in Niger and Senegal to develop locally-adapted guinea and non-guinea sorghum varieties and hybrids with improved grain quality and forage quality characteristics (e.g. tan-plant, white-grain, grain mold resistance, superior end-use characteristics … etc.).

3. Conduct proof of concept research on end-use grain quality and forage digestibility traits.

B. Linkage to Phase I objectives and activities

In phase I of the project, we used the sorghum genome sequence, a proven population of sequence-indexed mutants, and a large collection of genetically diverse sorghum accessions to identify and characterize allelic variation in genes that influence grain and forage quality traits. Once useful variants were identified, we initiated plant breeding populations to incorporate these traits into locally adapted cultivars and hybrids.

Phase 2 will be an extension of the successes of phase 1. We have already identified many novel mutations that have not been described before in sorghum. The proposed germplasm enhancement activities will result in technology transfer that contributes to development of sorghum varieties and hybrids with enhanced food- and feed-quality traits.

D. Training and outreach objectives

This proposed research, training, and capacity building project attempts to address key sorghum crop improvement needs through targeted research, short and long term training and education, and technology transfer to promote and enhance sorghum production. We intend to re-focus our capacity development efforts on the sorghum breeding programs in Niger and Senegal. Our priorities include a desire to establish and support the students trained in phase 1 as they transition into research scientists. We also intend to provide training and other support as needed for the breeding programs that we have helped establish in the region.

- We have established a protein digestibility lab in Senegal that can serve as a center of excellence for sorghum breeders across West Africa. We will work with ISRA/CERAAS to backstop and strengthen this program. We will also share genetic resources to strengthen efforts to develop new varieties with highly digestible protein.

- We intend to strengthen the sorghum breeding programs in Niger and provide special attention to the Forage Breeding program. We will work with INRAN to backstop and strengthen this program. If possible, we would like to establish a forage quality lab to support the forage breeder in the next phase of the project.

DEVELOPMENT OF BIOTIC STRESS-RESISTANT SORGHUM CULTIVARS FOR NIGER AND SENEGAL

1 – PRINCIPAL INVESTIGATOR

Bonnie B. Pendleton - *West Texas A&M University, USA*

2 – RESEARCH TEAM

Co-Investigators:

Lal K. Almas, Agricultural Economy - *West Texas A&M University, USA*
Gary C. Peterson, Sorghum Breeding - *Texas A&M AgriLife Research, USA* (retired 2018)
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M. Soumana Souley, Sorghum Breeding – *INRAN, Niger*
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William L. Rooney, Sorghum Breeder - *Texas A&M University, USA*
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Field and Laboratory Technicians - *INRAN, Niger*
Commercial seed companies

3 - PROJECT GOALS AND OBJECTIVES

1. Assist national sorghum programs in Niger and Senegal to develop biotic stress-resistant cultivars and inbred lines by crossing local or improved cultivars with known resistant sources

2. Provide a diverse array of germplasm potentially useful to research programs in Niger and Senegal

3. Better understand causes of sorghum resistance to sorghum midge, storage insect pests, and other biotic stresses

4. Develop a stored grain insect program to improve storage capability for high-quality grain

5. Analyze production profitability of improved sorghum cultivars using on-farm experiments and analyze potential marketing opportunities for use of sorghum and pearl millet in animal industry

6. Provide short-term and advanced degree programs (in the USA and/or Africa)

4) OVERVIEW OF ACTIVITIES

Seed of a diverse set of sorghum lines was increased and provided to collaborators in Niger and Senegal to evaluate for adaptation, grain yield potential, and reaction to biotic (insect and disease) and/or abiotic (drought) stresses in indigenous cropping systems. The lines were from the Texas A&M AgriLife Sorghum Improvement Program and represent genetic diversity in the breeding program for specific traits of interest. In Texas, more than 3,000 sorghum lines from Texas A&M AgriLife and commercial seed companies were evaluated in the seedling plant stage in greenhouse experiments for resistance against sugarcane aphid. The research led to identification of advanced lines resistant to sugarcane aphid in both the seedling and adult plant stages. Potential seed parents (A-/B-line pairs) were entered into a sterilization program to develop A-lines. R-lines were evaluated as hybrid male parents to determine combining ability and heterosis. The primary source of resistance to sugarcane aphids was derived from Tx2783, but several lines derive resistance from unknown sources. Exotic introductions with resistance as least as good as Tx2783 were identified. The lines were R-lines (restore fertility) in A1 cytoplasm, photoperiod insensitive, and three-dwarf height. Crosses were made to begin introduction of the resistance gene(s) into elite germplasm. From approximately 850 breeding lines evaluated, about 175 potentially resistant lines were identified. Eight exotic sorghum lines scored 1.0 to 3.0 as seedlings in the greenhouse and through maturity in the field and were released to the public. Damage starts when there are 50-125 aphids per plant, and 3,000-4,000 aphids per plant overwhelm the resistance. Evaluation of additional lines continued in the greenhouse and field.

In Niger, the project assessed the occurrence and diversity of storage insect pests of sorghum grain. The objectives of the research were to assess the occurrence of and storage insect pests infesting sorghum grain and determine diversity of storage insect pests by using multiple trapping techniques. Sorghum grain infested with pests was collected from storage facilities - 'Grinkan' at Bengou and 'SSD-35' and 'Mota Maradi' at Konni. Two samples of red and white sorghum were purchased from cereal markets at Niamey, Niger. Grain of each sorghum variety was kept in a laboratory and sieved every week to find and count insects that emerged. Three traps -- 'Dome' pheromone, sticky glue, and water bottle were used to monitor and determine diversity of insect pests in storage facilities. A taxonomic identification manual (Teetes et al. 1983) was used to identify the storage insect pests.

Scanning electron microscopy was used at Texas A&M University to determine that the mechanism of resistance to maize weevil, *Sitophilus zeamais*, was related to the distance from the outside of the seed coat to the starch in the aleurone layer of stored sorghum grain. Natural insecticide powders from four botanicals (neem, *Azadirachta indica* (leaves and bark); mesquite *Prosopis glandulosa* var. glandulosa; and milkweed, A*sclepias speciosa*) were evaluated for control of maize weevils in stored kernels of Malisor 84-7 sorghum from Mali (West Africa). Deterrence tests were done to evaluate feeding behavior of maize weevils on sorghum treated with botanicals compared to non-treated sorghum. Four local botanicals (neem kernels; fruit pulp of African locust bean, *Parkia biglobosa*; pericarp of *Hibiscus sabdariffa* fruit; and pulp of baobab fruit, *Adansonia digitata*) were evaluated for control of red flour beetle, *Tribolium castaneum*, in sorghum grain in Niger. In 2018, additional chemical analyses including high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy were used to determine the chemical composition of the botanical insecticides.

Rermetic bagging using five treatments (polyethylene bag (PE), clear plastic bucket (CPB), polypropylene bag (PP) (check) commonly used during storage in Niger, double bagging (DB) of one bag of (high-density polypropylene (HDPE) inside a polypropylene (PP) bag, and triple bagging (TB) of two high-density polypropylene (HDPE) bags inside a PP bag). Five newly emerged *T. castaneum* adults were put with sorghum grain in a container. Five newly emerged larvae of rice moth, *Corcyra cephalonica*, were put into a separate container.

<u>*Advanced Degree Education*</u>

The most qualified female and male students were recruited for graduate degrees, with the goal of one female and one male student. The students studied for graduate degrees at universities in Senegal and the United States. Our goal was exceeded. One female and one male from Senegal graduated with Masters degrees in Senegal in 2017. Two females from Senegal completed their Ph.D. degrees at a university in Senegal in 2018. After SMIL funding and TraiNet visa delays, a male scientist from Niger finally came to the U.S. in 2015, currently is working on his Ph.D. degree at West Texas A&M University, and is scheduled to graduate in May 2019.

During our SMIL project, our team of scientists and students gave 39 presentations; published 17 proceedings, 3 book chapters, and one refereed journal article; and completed and wrote two dissertations and two theses. The Ph.D. student from Niger who is studying at West Texas A&M University won four research awards, the West Texas A&M University undergraduate student worker assisting with SMIL research activities won an international entomological award, and one of the Ph.D. students from Senegal won an award for her research poster.

Short-term Training
Two female Ph.D. entomology students from Senegal participated in one-month short-term training in pest management, scanning electron microscopy and other techniques, and sorghum breeding at West Texas A&M University, Texas A&M University, and Texas A&M AgriLife Research at Corpus Christi and Lubbock during summer 2017.

Two-day workshops educated 94 stake-holders (including women) to identify and manage storage pests by turning advanced scientific knowledge and innovations into decision-making tools for maximum yield, quality, and healthy nutrition of sorghum at two regions in Niger and Senegal in 2016. At a one-day scientific conference in Niger in May 2018, 54 stake-holders were told our research results and informed how to manage insect pests of stored sorghum grain in West Africa.

5 - ACCOMPLISHMENTS

A. Achievements by Project Objectives

1. Assist national sorghum programs in Niger and Senegal to develop biotic stress-resistant cultivars and inbred lines by crossing local or improved cultivars with known resistant sources

Result completed - Germplasm developed through the program included sorghum midge-resistant cultivars for Niger and Senegal

Sorghum nurseries were grown to evaluate resistance to stresses at three locations in Niger in summer 2017 -- International Disease and Insect Nursery at Bengou, Midge Line Test (MLT) at Konni, and International Drought Line Test (IDLT) at Lossa. The IDIN and IDLT were provided to collaborators to select germplasm useful as parents in a sorghum breeding program.

The IDIN was developed to provide seed of standard checks and elite lines with genes for specific traits. Included in the nursery were germplasm lines resistant to biotic (insect, disease, and grain weathering/mold) and abiotic (drought - pre-flowering or post-flowering) stresses. For 2015-2018, the test included 25 entries and three replications. The IDLT contained standard checks and sources of resistance to either pre- or post-flowering drought. The entries were previously characterized for drought response. The standard checks included released germplasm lines from Texas and introductions from Africa. Experimental entries included germplasm lines from the defunct Sorghum Conversion Program and elite breeding germplasm. The test included 30 entries and three replications. The MLT was to evaluate germplasm for resistance to sorghum midge. Included were standard resistant and susceptible checks.

Provide a diverse array of germplasm potentially useful to research programs in Niger and Senegal

Result completed - Sorghum seeds from Texas A&M AgriLife Research at Lubbock were packaged and sent to Niger and Senegal for evaluating for resistance to biotic and abiotic stresses. Sorghum nurseries were planted at three locations in Niger and Senegal during summers of 2015 through 2018: International Disease and Insect Nursery (IDIN) at Bengou, Midge Line Test (MLT) at Konni, and International Drought Line Test (IDLT) at

Lossa. The IDIN and IDLT were provided to collaborators so each could select germplasm useful as parents in sorghum breeding programs.

Two different sources of resistance were included as checks. Experimental entries were selected for resistance and agronomic type in Texas. Most experimental entries could be developed as open-pollinated varieties – white grain and tan plants at least 1.5 m tall. Each year, the test had 50 entries and three replications. New lines in the MLT were added based on previous research to provide germplasm with potentially better agronomic adaptation and phenotype more relevant to indigenous cropping systems. For the IDIN at Bengou, Niger, BTx635, BTx631, BTx642, P954035, and Sureno were acceptable, but Segaolane was most damaged (score of 4). Damage to plants in the MLT at Konni, Niger differed between resistant and susceptible lines, with resistant check BTx3042 scoring only 1 and RTx640, MB108B, TAM2566, Tx2782, Tx2882, Tx2883, BTx378, and BTx643 scoring 2 on a scale of 1-5. Susceptible Tx2880 and three others scored 4. In the IDLT in Niger, potentially useful sorghum lines were NSA440, Tx2737, and Ajabsintido. The selected lines evaluated will be incorporated into a sorghum program for resistance to stresses in West Africa.

Better understand causes of sorghum resistance to sorghum midge, storage insect pests, and other biotic stresses

Result - Understanding the biology of sorghum midge in relation to daily flowering time of sorghum will enable development of more sustainable resistance. A Ph.D. student from Senegal did not do this planned activity in Senegal, but a Ph.D. student will do this planned activity to determine daily flowering time of spikelets of 10 sorghum genotypes in a field in Niger after he graduates in May 2019 and returns to resume his entomological activities at INRAN.

Result completed. Resistance of 10 sorghum genotypes and mechanisms of resistance were evaluated against two storage pests in Niger and Senegal. Six sorghum varieties were evaluated for resistance to *Tribolium sp.* and *Corcyra sp.* storage insect pests in Niger. The mechanism of resistance to an insect pest is seldom known and is almost impossible to determine. In complementary research, scanning electron microscopy was used at Texas A&M University to determine the mechanism of resistance to maize weevil in stored sorghum grain in Texas. The distance from the seed coat to the starch in the alleurone layer was 3.5 times greater in sorghum genotypes that were more resistant to maize weevils. Kernels of susceptible stored sorghum evaluated in a laboratory for resistance to storage insects weighed 80% less while resistant Seguifa and Sureno had lost less than 1% weight at 105 days after infestation by three female and two male maize weevils per vial of 5 g of sorghum grain.

Develop a stored grain insect program to improve storage capability for high-quality grain

Result - Practical and sustainable technologies were developed to enable long-term storage of healthy grain without use of pesticides.

Monitoring and assessing resistance of sorghum grain to storage insect pests. The objective of the research was to evaluate multiple trapping techniques to assess occurrence and diversity of storage insects in sorghum grain. Sorghum grain infested with storage pests were collected from storage facilities in Niger. Kernels of five sorghum varieties were kept in a laboratory and sieved every week to record insects that emerged. *Tribolium spp.* were dominant in all sorghum varieties sieved. *Corcyra cephalonica* adults were recorded only in the three improved varieties. *Sitophilus cerealella*, *Tribolium granarium*, and *Rhyzopertha dominica* were found in grain of red sorghum, and *S. cerealella*, *T. granarium*, and *R. dominica* adults were in white sorghum. Three traps were used to monitor and determine diversity of storage insect pests in storage facilities, and 10 species were trapped.

A Ph.D. student in Senegal found that after three months in storage, most adult *Tribolium* emerged from CE-180-33 sorghum. Fewest storage insects emerged from Sureno and Macia. Sureno and Macia were least damaged by *Ephestia kuehniella*.

Evaluating hermetic storage techniques to develop sustainable storage in West Africa. An experiment with five treatments evaluated hermetic bagging techniques to control *T. castaneum* and *C. cephalonica* in West Africa. The goal was to determine the best hermetic storage technique to control storage insect pests. Experiments were done in a laboratory in Niger. Treatments were a polyethylene (PE) bag, clear plastic bucket (CPB), polypropylene (PP) bag (check) commonly used during storage, double bagging (DB) by a bag of high-density polypropylene (HDPE) inside a PP bag, and triple bagging (TB) by two HDPE bags inside a PP bag. Five newly emerged *T. castaneum* adults were put with sorghum grain, as were five newly emerged *C. cephalonica* larvae put into a separate container. Sequential counts of dead adults were recorded every 7 days; live adults were counted at the end. The PP and DB killed most *T. castaneum* adults as well as most *C. cephalonica* adults. Most *T. castaneum* survived in the CPB. *C. cephalonica* adults survived in all but the PE bag.

Analyzing insecticidal properties of botanicals for managing insect pests of stored sorghum grain. The goal was to determine efficacy of selected botanical insecticides from plants to control storage insect pests in sorghum. Objectives were to assess mortality of maize weevils in Texas and red flour beetle in Niger, determine feeding inhibition by maize weevils presented sorghum treated with botanicals, and evaluate leaves and powder of each botanical to deter maize weevils. Experiments to assess efficacy of botanicals to control maize weevils were at the Entomology Laboratory at West Texas A&M University. Five treatments were a check (no plant material) and four powders of botanicals (neem leaves and bark, mesquite, and milkweed). To assess the number killed, five newly emerged maize weevil adults (two males and three females) were exposed to five doses (0.025, 0.05, 0.75, 0.1, and 0.2 g) of each plant powder applied to 5 g of sorghum grain. Numbers of dead maize weevils and other data were recorded every week for 35 days. To determine deterrence, leaves and powder of each plant were placed separately onto filter paper strips (1 × 1 cm) provided to maize weevils.

Experiments on effects of botanicals to control *T. castaneum* were done in a laboratory in Niger. Botanicals were neem kernels, African locust bean fruit pulp, pericarp of *H. sabdariffa* fruit, and baobab fruit pulp. Powder of each botanical at doses of 0.0125, 0.025, 0.05, 0.1, and 0.2 g were individually mixed with 5 g of sorghum grain, and the check had no plant powder. Five newly emerged *T. castaneum* adults were put into treated vials. Dead *T. castaneum* were counted every 7 days, and damage was scored on a scale of 1-5 for each botanical treatment per dose applied. Neem or African locust bean fruit pulp at ≤0.1 g killed >2.5 *T. castaneum* adults in sorghum grain. Pericarp of *H. sabdariffa* fruit and baobab fruit pulp at 0.2 g killed as many as 2.6 adults. Only 1.4 to 2.1 *T. castaneum* adults died with the check treatment. Damage scores were low for sorghum treated with >0.01 g of botanical powder compared to the check. Damage by *T. castaneum* was scored 3.3 in grain treated with 0.05 g of baobab fruit pulp.

All botanicals killed significant numbers of maize weevils compared to the check. Neem leaves at ≥0.5 g killed many weevils. Mesquite killed most weevils at doses of 0.25 and 0.5 g to sorghum grain. Milkweed powder killed more weevils at the greatest doses of 0.1 and 0.2 g. The powder detrimentally affected feeding by maize weevils on treated sorghum. Damage scores were 2.75 to 1.5 for sorghum grain treated with neem bark powder. Damage was great with scores of 2.0 and 2.75 for sorghum grain treated with milkweed powder at doses of 0.0125 and 0.25 g. For all sorghum kernels treated with powders at high doses of 0.1 and 0.2 g, damage was scored between 1.0 and 1.25. For the check, damage was greatest, with scores as much as 4.75.

Using a T-tube apparatus in a laboratory in Texas, powder of three botanicals (neem bark, mesquite, and milkweed) repelled male and female maize weevils, with a Repellency Index <1 (<1 repellent, 1 neutral, >1 attractant (Sakuma and Funkami 1985). At 30 and 45 minutes after exposure to treated sorghum grain, neem bark repelled 75.2 and 75.9% of female weevils, while mesquite repelled more males (70.3 and 76.2%), respectively. Milkweed-treated sorghum always repelled male weevils (70-75%).

Plant powders, especially at greater doses, deterred maize weevils from producing progeny. For neem leaf powder, most progeny developed at a dose of 0.5 g. As many as 3.25 maize weevil adults developed in sorghum grain treated with milkweed powder at doses of 0.0125 and 0.25 g. A mean of 6.75 maize weevils were produced in the check. Results from the studies confirmed effects of botanicals for controlling maize weevils attacking kernels of stored sorghum and other crops.

Four botanicals were evaluated for control of T. castaneum in sorghum grain in Niger. In Texas, natural insecticides from four botanicals were evaluated for control of maize weevil in stored kernels of Malisor 84-7 sorghum from Mali, West Africa. Three botanicals also were evaluated for control of maize weevil in stored kernels of Sureno.
Deterrence tests were done to evaluate feeding behavior of maize weevils on sorghum treated with botanicals compared to non-treated sorghum. In 2018, additional chemical analyses including HPLC and NMR were used to assess the chemical composition of the botanicals. Results are being analyzed and will be reported in the dissertation of the Niger Ph.D. student studying at West Texas A&M University.

Farmers will learn to identify and manage pests of stored sorghum grain. In 2016, a total of 94 stake-holders including women were educated to identify and manage pests of stored sorghum grain by turning advancement of scientific knowledge and innovations into decision-making tools for maximum yield, quality, and healthy nutrition at two regions in Niger and Senegal. In 2016, 94 stake-holders were educated at two-day workshops in West Africa. At a one-day scientific conference on 30 May 2018 in Niger, 54 stake-holders were told our research results and informed how to manage insect pests of stored sorghum grain in West Africa.

Analyze production profitability of improved sorghum cultivars using on-farm experiments and analyze potential marketing opportunities for use of sorghum and pearl millet in animal industry

Result ongoing - will be completed in Phase II.

An experimental protocol was developed to collect data on production profitability of on-farm experiments with our sorghum midge-resistant sorghum for economic analysis of improved sorghum storage techniques in West Africa. Production data will be organized as input costs and output benefits and analyzed for improved sorghum varieties released to farmer associations in West Africa.

Proactive steps were taken to account for gender-specific constraints, preferences, and practices in project activities to assure proportional participation and input from female and male farmers and consumers.

Result completed. Education and training sessions allowed male and female participation. Women brought their "young farmers" to short-term training workshops. Educational materials were adapted to the varying literacy levels of participants.

Result completed. Female and male candidates for degree education were given equal opportunities. The goal of one female and one male student graduating with advanced degrees was exceeded. One female and one male from Senegal graduated with Masters degrees in Senegal in 2017. Two females from Senegal completed Ph.D. degrees in 2018. A male scientist from Niger currently working on his Ph.D. degree at West Texas A&M University is scheduled to graduate in May 2019.

Result – to be completed in Phase II. Women and men farmers will be consulted for assessment of plastic bags and hermetic containers for storing grain. The activity is being tested in a laboratory and data are being analyzed to determine the appropriate technology for demonstration sessions with farmers.

B. Project Major Challenges and Adjustments

Late sub-contracting that was delayed for 6 months after the start of SMIL and insufficient funding during that time prevented realization of some proposed activities during the first year of the project. From the beginning, SMIL principal investigators were not permitted to spend funding on educational or research activities on behalf of the U.S. The SMIL Management Entity always was slow in informing us and requesting project reports and other relevant documents with short deadlines.

The TraiNet process to obtain visas for students from other countries to study in the U.S. delayed beginning an advanced degree for 10 months. A scientist from Niger who planned to study agricultural business and economics could not pass the TOEFL to be admitted for a Masters degree at West Texas A&M University. In 2016, two Ph.D. students from Senegal delayed obtaining their visas until it was too late for sorghum to be in the field in summer 2016, so the students finally obtained visas to come to the U.S. in summer 2017. The two students also until did not speak English well enough to thoroughly understand sorghum breeding and pest management research techniques being explained to them during their one month of short-term training in summer 2017.

In Senegal, research focus on sorghum resistant to sorghum midge to determine the daily time when midges were in fields and flowering time of spikelets was diverted to recording data on sorghum resistance to sorghum midge, diseases, and other factors. The INRAN cereal entomologist while earning his Ph.D. at West Texas A&M University was not available to determine the daily time sorghum midges are in sorghum fields and spikelets flower in West Africa, but will do the activity when he graduates and returns to Niger.

In Niger, the DHL shipping company did not deliver seeds on time because of their incomprehension of customs fees to be charged before delivering packages of seeds. This delayed planting of the sorghum seeds at the research stations in Niger in 2017.

Continuous power shortages in Niger in 2017 and 2018 hindered survival of *Tribolium castaneum* and *Corcyra cephalonica* colonies being reared for use in evaluating hermetic bagging and botanical insecticides to manage insect pests in stored sorghum grain. Power shortages during the research period (summer) hindered weighing of samples damaged by storage pests. Power shortages hindered maintenance of *Tribolium* and *Corcyra* colonies needed for economic analysis on improved storage sorghum techniques in Niger. Frequent power shortages also delayed and hindered the scientific conference in Niger in 2018.

C. Student Training Achievements

Results of Objective 6 of our project to provide advanced degrees in the U.S. and Africa are almost complete and will be finished in May 2019. Females and males studied for graduate degrees in Senegal and the U.S. The INRAN cereal entomologist who is at West Texas A&M University did an excellent job on written and oral preliminary exams for his Ph.D. and advanced to candidacy in March 2018 and will complete his degree and graduate in May 2019. A male student in Senegal completed his M.S. degree and thesis entitled "Evaluation of sorghum lines for tolerance to insects and disease in the groundnut basin area" in December 2015. One female and one male from Senegal graduated with Masters degrees in Senegal in 2017. Their theses were entitled "Evaluation de la resistance aux maladies et insects ravageurs de lignees de sorgho texanes (*Sorghum bicolor* (L.) Moench) en rapport avec leurs cycles de developpement dans la zone de Nioro du RIP" and "Dynamique des populations de la cecidomyie du sorgho *Stenodiplosis sorghicola* (Diptera: Cecidomyiidae) et cycle de developpement des lignees de sorgho (*Sorghum bicolor* (L.) Moench dans la zone de Roff (Mbour)." Two females from Senegal completed Ph.D. degrees in 2018. The dissertation of Adja Nene Thiam was entitled "Evaluation de la resistance a la cecidomyie de divers genotypes ou varieties de sorgho et effect de la periode de floraison sur leaur tolerance au Senegal." Fatou Welle researched stored grain pests with her dissertation entitled

"Evaluation de la resistance varietale et de methods alternaties pour la gestion des principaux insects des stocks du mil/sorgho au Senegal."

During this SMIL project, our team including scientists and students gave 39 presentations; published 17 proceedings, three book chapters, and a journal article; and wrote two dissertations and two theses. The Ph.D. student from Niger who is studying at West Texas A&M University won four research awards, a West Texas A&M University undergraduate student worker assisting with SMIL research activities won an international entomological award, and one of the Ph.D. students from Senegal won an award for her research poster.

D. Short-term Training and Outreach

Objective 6 of our project provided short-term training in the U.S. and Africa. Two female Ph.D. entomology students from Senegal participated in one-month short-term training in sorghum pest management, electron microscopy techniques, and breeding at West Texas A&M University, Texas A&M University, and Texas A&M AgriLife Research at Corpus Christi and Lubbock in summer 2017. At Texas A&M University, College Station both students learned techniques to evaluate sorghum grain with a scanning electron microscope. Methodology to prepare and analyze different sorghum grains was taught. Research at West Texas A&M University involved methodology to evaluate sorghum genotypes for resistance to maize weevils of stored sorghum grain. Evaluation for resistance to sugarcane aphid included rearing aphids, planting seeds, infesting seedling plants, and scoring plant damage by sugarcane aphids. Sugarcane aphid biology was evaluated in clip cages on sorghum in an incubator. Plots of sorghum evaluated for resistance to biotic and abiotic stresses including sorghum midge and sugarcane aphids were evaluated at Texas A&M AgriLife at Corpus Christi. Research methodology used in a multi-location breeding program was discussed. Objectives of the breeding program, multi-location evaluation, and distribution of lines to international cooperators were discussed. At Texas A&M AgriLife at Lubbock, seeds were packaged and prepared for planting locally and for distribution to collaborators. The intention of the training program was to 1) increase awareness of and expertise in methodologies used in collaborative research and 2) demonstrate how scientists in diverse disciplines work to understand and solve problems.

Host-country farmers, including women, were taught to use information from the project for better sorghum production. In 2016, 94 male and female stakeholders were educated to identify and manage pests of stored sorghum grain in Niger and Senegal. Modules discussed included identification, biology, and management of storage pests, scanning electron microscopy, and techniques to monitor quality of stored sorghum grain and seeds. A video was produced of the two-day workshop in Niger. In May 2018, 54 stake-holders in Niger participated in a scientific conference to learn research progress made on our SMIL-funded activities and were taught to manage insect pests of stored sorghum grain. Multiple videos were recorded of the scientific conference.

6 - UTILIZATION OF RESEARCH OUTPUTS

A. Technologies and Management Practices by Phase of Development

The International Disease and Insect Nursery, Midge Line Test, and International Drought Line Test were provided to West African collaborators and evaluated for resistance to biotic and abiotic stresses for use in sorghum breeding programs. In Phase II, the sorghum lines selected during Phase I will be incorporated into sorghum germplasm and used for future improvement of sorghums for resistance to biotic and abiotic stresses in West Africa. The resistant sorghum lines were selected based on agronomic characteristics, resistance to stresses, and adaptation and phenotype relevant to indigenous cropping systems.

Resistance of sorghum genotypes and mechanisms of resistance were evaluated to storage pests in Niger and Senegal. Six sorghum varieties were evaluated for resistance to *Tribolium sp.* and *Corcyra sp.* storage insect pests in Niger. Scanning electron microscopy was used at Texas A&M University to determine mechanisms of resistance to maize weevil in stored sorghum grain in Texas.

A water-bottle trap can be used to monitor insect pests in storage facilities the farm level. Polypropylene bag and double bagging consisting of a bag of high-density polypropylene inside a polypropylene bag were the most effect hermetic storage techniques to manage *T. castaneum* and *C. cephalonica* in sorghum.

Neem leaves and mesquite powders at ≥0.5 g killed most maize weevils in sorghum grain in Texas. Damage scores were 2.75 to 1.5 for grain treated with neem bark powder. Neem kernel and African locust bean fruit pulp killed more *T. castaneum* in a laboratory in Niger. Damage scores were low for sorghum treated with >0.01 g of botanical powder compared to the check.

Data on production profitability of improved cultivars used on-farm will be organized as input costs and output benefits and analyzed for improved sorghum varieties released to farmer associations in West Africa.

B. Intermediate Outputs

For the International Disease and Insect Nursery, BTx635, BTx631, BTx642, P954035, and Sureno were acceptable, but Segaolane was most damaged in Niger. Damage in the Midge Line Test differed between resistant and susceptible lines, with check BTx3042 most resistant; RTx640, MB108B, TAM2566, Tx2782, Tx2882, Tx2883, BTx378, and BTx643 moderately resistant; and Tx2880 most damaged. In the International Drought Line Test, NSA440, Tx2737, and Ajabsintido were best in Niger. The sorghum lines will be used for future studies on resistance to stresses.

Activity to understand the biology of sorghum midge in relation to daily sorghum flowering time to enable development of more sustainable resistance will be done during Phase II in Niger.

A water-bottle trapping technique will be used at the farm level to monitor insect pests in storage facilities during Phase II. A polypropylene bag and double bagging by a bag of high-density polypropylene inside a polypropylene bag will be evaluated against other storage techniques to manage storage pests in sorghum at the farm level during Phase II.

Botanical insecticides neem leaves and kernels and African locust bean fruit pulp can be used to effectively control storage pests in sorghum without relying on chemical insecticides. Botanicals will be evaluated against other pests at the farm level during Phase II.

More workshops and field days will be scheduled during Phase II to teach stake-holders new technologies to manage sorghum insect pests.

7 - PROPOSED FUTURE ACTIVITIES FOLLOWING PHASE I ACCOMPLISHMENTS

A. Statement on Future Activities in Phase II

In the U.S. and West African countries where this project was implemented during Phase I, major insect pests of sorghum in the field and storage continue to seriously constrain quantity and quality of grain. Sorghum midge is the most damaging insect pest of sorghum worldwide, including in the Americas, Africa, and Australia. The sugarcane aphid invaded and decimated sorghum in the southern U.S. in 2013, and the new exotic species of fall armyworm, *Spodoptera frugiperda*, infested more than 30 crops in almost all countries on the African continent beginning in 2014. Globally and mostly in developing countries, storage insect pests continue to cause major damage to stored products. The following provides a short review of major impacts of these insect pests on sorghum and the need for more and continuing integrated pest management research especially on pest-resistant sorghums.

Sorghum is the 5th major cereal crop worldwide, especially in semi-arid tropics. Sorghum provides food, feed, and forage, but grain yields at the farm level are low, partly because of damage by insect pests (FAO and ICRISAT 1996). Sorghum is a staple for the poorest and most food-insecure people in the world. About 150 species of insect pests damage sorghum. Sorghum midge is the most important pest in all sorghum-growing regions of Africa, the Americas, Asia, and Australia. In many parts of the world where sorghum midge is endemic, progress has been made in development and application of various management methods. Often, control relies on chemical treatment, use of local "know-how", or new technologies to manage pests. Although success has been achieved in applying methods to control sorghum midge in many areas, the sorghum midge still is a serious constraint to production, and sustainable control methods including resistant sorghums need to be developed for use at farm level. Research is designed to study interrelationships between sorghum midge and plants in the field.

Fall armyworm is a moth indigenous throughout the Americas where it is one of the most damaging insect pests, feeding on more than 80 crops including sorghum, maize, rice, and sugarcane, as well as millet, alfalfa, beet, cabbage, cotton, groundnut, onion, pasture grasses, potato, soybean, and tomato. It previously was only in the Americas but now has become established in Africa and rapidly spread throughout tropical and subtropical regions of the continent. Fall armyworm can migrate long distances on prevailing winds but also breed continuously in areas with suitable weather (Day et al. 2017). There is a large volume of literature on control of fall armyworm in the Americas, but the agricultural systems often are very different from those in Africa. New IPM techniques that do not rely on insecticide and are compatible with climate and sorghum growth and development are needed to control fall armyworms in sorghum in West Africa.

The sugarcane aphid first infested sorghum along the Texas Gulf Coast and Louisiana in 2013 and significantly damaged yield and affected harvest efficiency by abundant honeydew (Villanueva et al. 2014). In 2015, the aphid was on grain sorghum, sorghum-sudan hybrids, sweet sorghum, millet, and Johnsongrass in more than 400 counties in 17 states on the Great Plains of the U.S. and in all sorghum-producing regions in Mexico (Bowling et al. 2015). Sugarcane aphid identification, monitoring, and treatment guides were developed for use of insecticide (Bowling et al. 2016), but environmentally friendly, non-chemical pest management techniques including resistant sorghums are needed. Public and private sorghum breeding programs including entomologists and breeders involved with the project in the U.S. quickly developed and evaluated sorghums resistant to the sugarcane aphid.

Beetle and moth pests destroy 5-35% of stored grain worldwide, but destroy 40% or more in developing countries of the tropics and subtropics (Schulten 1975). Weevils are the most destructive insect pests of stored grain including sorghum in the U.S. and worldwide, especially in warm, humid countries (Teetes 1983). Adult rice weevil, *Sitophilus oryza*, and maize weevils are 4 mm long and reddish brown. A female maize weevil lays 300-400 eggs on kernels in the field or storage, and larvae developing inside destroy the kernels. Plants with insecticidal properties have been used traditionally for generations throughout the world. Botanicals are particularly relevant during post-harvest storage of commodities by small-scale farmers (Proctor 1994, Dales 1996). In developing countries, botanicals have advantages over synthetic pesticides because they are gathered locally and provide inexpensive pest control during storage.

This multidisciplinary program will be a continuation of Phase I of our project and cover activities not fully completed in Phase I. During Phase I of our project, new invasive species (sugarcane aphid and fall armyworm) infested and destroyed sorghum and other crops in our areas of research in Africa and the U.S. Phase II will include new research activities on sorghum resistance to manage insect pests in storage and in the field including sorghum midge, fall armyworm, and sugarcane aphid.

1. *Understanding the biology of sorghum midge in relation to sorghum flowering time will enable development of sorghum with more sustainable resistance*

   Objective: Better understand causes of sorghum resistance to sorghum midge, storage insect pests, and other biotic stresses.

   Key Activities:

   - Initiate a program to breed new sorghum midge-resistant lines by using the resistant genotypes identified through Phase I evaluation activities
   - Determine flowering time of spikelets of sorghum genotypes developed for resistance to sorghum midge at one location
   - Determine the daily time sorghum midges are in sorghum fields at locations where midge in a major constraint in Niger
   - Use field training to educate male and female stakeholders on identification and management of sorghum midge

2. *Research activities on management of storage insect pests*

   Objective: Develop sustainable techniques to control storage insect pests of sorghum grain

   Key Activities:

   - Identify and evaluate alternative management techniques to control storage insect pests
   - Study effects of inert substances to control storage insect pests
   - Isolate botanical extracts and/or oils to reduce damage by storage insect pests on sorghum
   - Determine best hermetic bagging techniques through on-farm research

3. *Research activities to identify integrated pest management techniques to control fall armyworm in West Africa*

   Objective: Use plant diversity to develop an integrated pest management package for control of fall armyworm in sorghum

   Key Activities:

   - Identification and application of compatible control methods to control fall armyworm in sorghum
   - Identify key hosts of fall armyworm and determine the amount of damage on sorghum
   - Evaluate sorghum genotypes to identify sources resistant to damage by fall armyworm
   - Use plant diversity, such as push-pull strategy to manipulate distribution and abundance of fall armyworm by combining behavior-modifying stimuli

- Initiate and implement an educational program on fall armyworm identification, damage, and integrated pest management techniques and application for sorghum stakeholders (farmers, extension agents, and others).

4. *Research activities to study biology and plant resistance to control sugarcane aphid in the United States*

Objective: Expand knowledge of sugarcane aphid biology and identify sorghum lines resistant in the U.S.

Key Activities:

- Evaluate sorghum genotypes to identify sources resistant to damage by sugarcane aphids in the field and greenhouse

- Study effects of photoperiod and temperature on fitness and biology (longevity, fecundity, etc.) of sugarcane aphid development in a laboratory

B. Linkage to Phase I Objectives and Activities:

In the U.S. and West African countries where the project was implemented during Phase I, major insect pests of sorghum in the field and storage continue to seriously constrain quantity and quality of sorghum grain. Sorghum midge is the most damaging insect pest of sorghum worldwide including throughout Africa. The sugarcane aphid invaded and decimated sorghum in the southern U.S. in 2013, and the new exotic species of fall armyworm infested more than 30 crops in almost all countries on the African continent beginning in 2014. Globally and mostly in developing countries, storage insect pests continue to cause major damage to stored products.

This multidisciplinary program will be a continuation of Phase I of our project and cover activities that were not fully completed in Phase I. It also will include new research activities on sorghum resistance against fall armyworm in Africa and sugarcane aphids in the U.S. In Phase II, we propose research activities for sorghum resistance to control insect pests including sorghum midge, fall armyworm, and sugarcane aphid in the field and greenhouse, and storage insects.

- Better understanding the biology of sorghum midge in relation to sorghum flowering time proposed to be done in Phase I will be done in Phase II.

- Research activities on management of storage insect pests stated in Objective 4 of Phase I will be expanded in Phase II to include studying effects of inert substances and isolating botanical extracts and/or oils to reduce damage by insect pests of stored sorghum grain.

- Evaluation of sorghums newly developed for resistance to sugarcane aphids will continue during Phase II. Knowledge of photoperiod and temperature on biology and fitness (longevity and fecundity) of sugarcane aphid in a laboratory will expand during Phase II so sorghums with more sustainable resistance can be developed for use in Africa and the U.S.

- Following invasion by fall armyworm throughout much of the African continent, research activities in Phase II will identify integrated pest management techniques. Key hosts of fall armyworm and damage to sorghum will be determined. Genotypes will be evaluated to identify sources of sorghum resistant to fall armyworm. Plant diversity, such as a push-pull strategy, will be used to manipulate distribution and abundance of fall armyworm.

- Advanced degree education and short-term training will continue in Phase II for human capacity building of scientists and sorghum stake-holders in African countries. In Phase II, a research and development program will be initiated and implemented to transfer innovations/techniques from Phase I such as sorghum genotypes resistant to biotic stresses (sorghum midge, sugarcane aphid, and fall armyworm), and water-bottle trapping, botanical insecticides, and hermetic bagging to manage storage insect pests.

C. Training and Outreach Objectives

1. *Advanced education for M.S. and Ph.D. degrees in U.S. and/or African universities*

Objective: Provide advanced degree education at African and U.S. universities

Key Activity:

- Identify potential candidates and educate M.S. and Ph.D. students at African and U.S. universities

2. *Short-term training of research technicians, extension agents, and farmers*

Objective: Develop and do short-term training for stakeholders to control major insect pests of sorghum

Key Activities:

- Develop educational modules on key insect pests of sorghum
- Provide training through workshops, field days, and training-and-visits

3. *Transfer of technologies developed in Phase I*

Objective: Initiate and implement a research and development program to transfer innovations/techniques developed during SMIL Phase I

Key Activities:

- Determine potential partners for a research and development program
- Identify farmers and/or farmers' organizations to be included in the research and development program
- Implement transfer of innovations/techniques at the farm level
- Report progress and completion of all the training and outreach programs

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DEVELOPMENT OF DUAL-PURPOSE PEARL MILLET VARIETIES FOR THE BENEFIT OF FARMERS AND AGRO-PASTORALISTS IN THE SAHELIAN AND SUDANIAN ZONES OF WEST AFRICA

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3 - PROJECT GOALS AND OBJECTIVES

The overall, long-term goal of the present project is to contribute to increasing pearl millet smallholder farmers' income, nutrition and resilience in Niger, Burkina Faso, Mali and Senegal through participatory development of dual purpose, highly nutritious pearl millet varieties.

The specific objectives achieved are:

1. To gather and screen the genetic diversity of West African pearl millet germplasm (landraces and breeding materials) for stover quality/digestibility traits and grain mineral content;

2. To assess relationships between stover nutritional quality and digestibility and other agro-morphological traits including grain mineral contents, to understand potential trade-offs in selection of dual purpose, nutritious pearl millet cultivars;

3. To validate and to select the 15 superior germplasm accessions identified under specific objective 1 together with women and men farmers in large-scale on-station and on-farm trials in the target countries;

4. To identify the 3 to 5 farmer-preferred accessions for future promotion and use in pearl millet dual purpose variety development;

5. To develop a longer-term strategy for breeding highly nutritious dual-purpose pearl millet cultivars in West Africa.

4 - OVERVIEW OF ACTIVITIES

To achieve this goal, each one from the four target countries and ICRISAT contributed the 15 to 20 best varieties that they thought were good for grain and fodder productivity. A set of one hundred accessions was created (Table 1 in annex). These materials are composed of local landraces, local populations, improved varieties and hybrids.

The materials were tested in two repetitions by site into the two agro ecological zones (soudanian and sahelian) in each of the four countries. The fertilization rate and the management practices were done according to the country recommendations for millet cultivation.

Senegal

In Senegal, the distance between rows was 90 cm and between plants on the row is 90 cm. Each plot was composed of two rows of 6.30 cm length or 16 plants. Each plant was thinned to 2 plants per pocket.

The fertilization rate was 150 kg/ha of NPK 15-10-10 during land preparation and 100 kg/ha of urea divided into 2 applications, the first after thinning and the second after second wedding.

To achieve these different goals:

1. We processed by gathering the best genetic materials for grain and fodder production from the different countries involved (Burkina Faso, Mali, Niger and Senegal). One hundred (100) accessions have been collected.

2. We conducted on station characterization trials on Sahelian and Soudanian zones in each of the 4 countries. These actions have been done during two years (2015 and 2016) and from the results and the farm evaluation; we selected the best 15 accessions for each agroecological zones.

For Senegal, the best accessions selected by agroecological zone were tested on station and on farmers' fields. The trial was conducted at Bambey station for the Sahelian zone and Nioro station for the Soudanian zone.

Results of Sahelian Zone:

The results showed that the most high grain yielding variety in Sahelian zone is SL214 (2,768kg/ha). It is followed by SL442 (2,691kg/ha) and Thialack2 (2,625kg/ha). For stover production, the best variety is SL106 with 6,585kg/ha, followed by SL214 with 6,330kg/ha.

For the grain analysis, the richest variety for iron is NK Moro from Niger. There were also SL69, SL56 from Senegal; IBMV 8402 et PE01203xPE05980R3 that showed a high level of iron content.

For fodder analyses, we observe that the highest ADF content belongs to SL214 with 50.55. This variety has also a medium level of NDF (74.72) and a good rate of protein content (3.48). This variety is followed by PE08030 from Burkina Faso with 50.25 as ADF, 76.10 as NDF and 3.75 as protein content. After these two elite varieties, we have Thialack2 with 49.51 as ADF, 73.63 as NDF and 5.86mg/kg as protein content (Table 2).

Conclusion: These Senegalese results for the Sahelian zone showed that for a next step of dissemination, these 3 varieties (SL214, PE08030 and Thialack2) are well positioned for on farm cultivation.

Table 2: Characteristics of the 15 selected varieties for the on farm Sahelian zone

DONNEES PASSEPORT			AGROMORPHOLOGICAL					GRAIN ANALYSES		FODDER ANALYSES			
N°	Pedigree	Pays	HTR	FLO	LEP	PTIG	RDT kg/ha	Fer	Zinc	ADF MOY	NDF POY	RFV MOY	Protein MOY
1	BF PE08030	SENEGAL	226	50	52	4705	2018	33,4	30,3	50,25	76,10	60,83	3,75
2	PE08011	BURKINA	248	51	55	3535	2260	31,5	28,4	46,68	71,39	68,56	4,62
3	BF PE01203XPE05980R3	BURKINA	265	51	58	4730	2269	32,5	28,3	47,81	72,16	66,69	3,82
4	IBMV8402	SENEGAL	231	49	41	5650	2190	33,0	28,2	45,89	71,14	70,07	5,49
5	NG HK Moro	NIGER	252	50	45	4710	2193	33,7	33,7	47,41	70,73	68,37	3,23
6	SL516	SENEGAL	263	54	66	5030	2211	31,1	30,6	48,70	74,12	63,97	3,07
7	SL 56	SENEGAL	238	53	50	5065	2171	32,1	30,4	46,74	72,28	67,57	4,30
8	SL 69	SENEGAL	239	49	48	5330	2116	32,6	27,1	41,33	64,92	81,25	3,52
9	SL 328	SENEGAL	279	51	61	5170	2177	30,9	35,3	49,30	73,64	63,79	5,53
10	SL 167	SENEGAL	234	51	51	4495	2360	31,5	30,1	46,44	70,49	70,09	5,18
11	SL 214	SENEGAL	260	54	42	6330	2768	31,7	30,7	50,55	74,72	61,68	3,48
12	SL 106	SENEGAL	240	50	57	6585	2215	34,9	29,3	45,87	70,34	70,71	3,82
13	SL 45	SENEGAL	232	50	43	4195	2116	32,3	31,1	49,28	73,24	64,15	4,22

14	SL 442	SENEGAL	242	48	42	5420	2691	28,8	27,5	47,45	70,85	68,53	3,31
15	THIALACKZ	SENEGAL	253	52	66	5200	2625	30,5	34,1	49,51	73,63	63,61	5,86

Results for Soudanian Zone

The Soudanian zone results are shown in table 3 below. The results showed than there is a significant difference between entrees for all the variables calculated. In addition, some varieties are significantly better than other for the main traits.

For grain yield, the results showed that ICMVIS89305 is the more productive variety with 2.458t/ha. It is followed by GA-HKP from Niger and SL49 from Senegal. They produced respectively 2.196t/ha and 2.190t/ha.

For fodder production, the more productive variety is PE0967C0 from Burkina Faso with 9.45t/ha. After that one, we have PE000437xPE00273 from Burkina Faso with 8.75t/ha, NKO/TC1 from Mali with 8.25t/ha, Groupe7 from Burkina Faso with 7.95t/ha and SL49 with 6.25t/ha.

The biochemical analyses showed that GBxMoro is the richer variety for iron containing 39.90 mg/kg. Just following is NKO/TC1 with 39.80mg/kg and PE00967 with 39.50mg/kg. For Zinc content, we noticed that there two (2) varieties from Mali that are the highest. They are CzSyn00-02 and NKO/TC1 with 33.1mg/kg. The most important Senegalese iron and zinc content variety is SL170 with 33.80mg/kg for iron and 27.90mg/kg for zinc of dry matter.

For fodder analyses, we observed that the most important variety for ADF content is NKO/TC1 with 45.01mg/kg of dry matter. The NKO/TC1 has also a medium level of NDF which is 68.15 and a high level of protein contain that is 5.36mg/kg of dry matter. Following are three other important varieties that are Groupe7 from Burkina Faso with 44.76mg/kg, CzSyn00-02 with 44.59mg/kg and ICMVIS 89305 with 43.04 as ADF. The levels of protein for these varieties are respectively 4.52mg/kg, 5.97mg/kg and 4.32mg/kg.

Conclusion: the results showed that NKO/TC1 and CzSyn00-02 have an important iron and zinc content but their duration is too long for Senegalese Soudanian zone. They may not mature during medium and short rainy seasons. Similarly, PE00967 and Groupe7 are very productive with fodder but their panicle length is very short for farmers in Senegal. The farmers may not adopt these types of millet varieties. Therefore, for the Soudanian zone in Senegal, the most adapted varieties for dual-purpose proprieties are GA-HKP from Niger, ICMVIS 89305 from ICRISAT and SL170 from Senegal.

Table 3: Characteristics of the 15 selected varieties for on-farm testing in the Soudanian zone

DONNEES PASSEPORT			AGRONOMICAL DATA					GRAIN ANALYSES		FODDER ANALYSES			
N°	PEDIGREE	PAYS DE PROVENANCE	FLO	HTR	LEP	PTIG	RDT kg/ha	Fer	Zinc	ADF	NDF	RFV	Protein
1	PE05578 C1	BURKINA	56	273	41	5550	1612	35,00	30,70	42,21	64,56	80,71	6,15
2	PE00967 C0	BURKINA	66	132	48	9450	359	39,50	30,00	39,19	60,31	90,13	6,25
3	Groupe7	BURKINA	58	293	38	7950	474	36,10	28,30	44,76	64,73	77,70	4,52
4	GBxMORO	BURKINA	49	225	36	4750	2060	38,90	25,20	41,88	63,39	82,67	5,45
5	PE00437XPE00273	BURKINA	58	274	36	8750	1356	35,10	26,60	42,73	68,66	75,42	5,47
6	ICMVIS89305	SENEGAL	52	292	46	6600	2458	28,50	22,80	43,04	66,75	77,17	4,32
7	CzSyn 00-02	MALI	57	310	43	6700	1996	32,80	33,10	44,59	67,24	74,94	5,97
8	NKO/TC1	MALI	64	259	42	8250	621	39,80	31,10	45,01	68,15	73,64	5,36
9	PEO6001	NIGER	54	210	35	3750	789	28,80	24,30	37,83	61,68	89,85	8,27
10	PE05346	NIGER	59	243	42	7750	1243	37,80	31,80	39,49	61,86	87,84	5,27
11	GA-HKP	NIGER	53	282	46	6025	2196	30,10	26,80	40,72	65,03	82,35	5,43
12	SL-49	SENEGAL	52	221	50	6250	2190	31,60	23,60	41,68	62,70	83,80	5,78
13	SL-69	SENEGAL	49	294	39	5000	574	31,90	26,10	42,77	63,42	81,62	3,29

14	SL-328	SENEGAL	54	249	64	3950	1853	30,00	24,10	43,11	65,15	79,01	5,95
15	SL-170	SENEGAL	51	239	41	5400	1329	33,80	27,90	42,07	63,39	82,37	4,25

Burkina Faso

Results for the Sahelian Zone

For Burkina Faso, the trials in Sahelian zone were conducted at Katchari (Dori) research station. One hundred accessions from the four countries were evaluated in 2015 and 2016. Data from evaluation allowed the selection of 15 best accessions on the basis grain yield and fodder yield. The grain and fodder quality analysis were performed for the selected accessions. Results are presented in Table 4 below.

Table 4 : Agronomic performance of selected accessions for Sahelian zone

Entry	Designation	Agronomic performance							
		FLO	HPM	LOE	DAE	GsE	HI	GY	STY
2	**KalpelgaxPE03922**	80	221	31	2,6	51	38	1191	3320
3	**PE0437xPE00515**	81	205	40	2,7	64	42	1279	3086
11	**Sahel 22 C0**	81	215	37	2,7	53	41	1367	3359
17	**PE02987 X ICMV IS 92222**	65	200	47	2,3	58	47	1113	2930
18	**PE02853xPE00404 R3**	83	221	61	2,0	57	45	1064	2930
23	**Groupe7**	78	209	29	2,0	58	29	1025	3047
29	**PE00397XPE00404**	81	205	39	2,3	53	50	1318	3086
30	**PE02987XPE05347**	65	192	37	2,1	37	39	840	3594
36	**SoSat**	81	203	35	3,1	59	32	1172	3516
41	**CzToroniou HTC Aristé**	81	210	33	2,2	69	64	2002	3516
46	**Djiguifa**	63	203	45	2,2	50	33	986	2930
47	**CzMil Aristé**	65	189	34	2,5	54	44	1074	2617
72	**GAMOGI**	77	191	40	3,1	53	45	1367	3125
86	**S1-214**	76	211	52	2,0	57	47	1465	3477
97	**S1-442**	82	191	45	2,0	54	50	1084	2734
	Minimum	*63*	*189*	*29*	*2,0*	*37*	*29*	*840*	*2617*
	Maximum	*83*	*221*	*61*	*3,1*	*69*	*64*	*2002*	*3594*
	Average	***76***	***204***	***40***	***2,4***	***55***	***43***	***1223***	***3151***

Table 5: Grain and Fodder quality of selected accessions for Sahelian zone

Entry	Designation	Grain quality		Fodder quality			
		Fer (mg/kg)	Zinc (mg/kg)	ADF	NDF	RFV	Protein
2	KalpelgaxPE03922	32,0	33,4	42,6	64,0	82,7	2,5
3	PE0437xPE00515	44,2	38,4	43,4	64,9	79,1	4,0
11	Sahel 22 C0	37,3	37,1	44,8	67,4	74,6	4,2
17	PE02987 X ICMV IS 92222	30,1	33,2	40,4	61,0	88,4	6,5
18	PE02853xPE00404 R3	30,9	30,1	43,4	64,6	79,7	3,6
23	Groupe7	33,4	29,3	42,9	63,2	81,7	3,6
29	PE00397XPE00404	31,7	28,2	42,0	63,5	82,3	4,1
30	PE02987XPE05347	34,8	36,7	40,2	61,1	88,0	3,0
36	SoSat	29,2	28,9	39,0	59,0	92,3	3,3
41	CzToroniou HTC Aristé	35,6	36,6	40,2	60,7	88,3	3,7
46	Djiguifa	40,5	36,4	42,0	63,0	83,4	4,3
47	CzMil Aristé	33,8	27,6	40,3	61,4	87,5	3,8
72	GAMOGI	37,2	34,7	41,4	61,3	86,5	3,8
86	S1-214	32,3	35,1	40,0	59,7	90,0	3,5
97	S1-442	34,8	39,2	46,7	65,9	74,3	3,1
	Minimum	*29,2*	*27,6*	*39,0*	*59,0*	*74,3*	*2,5*
	Maximum	*44,2*	*39,2*	*46,7*	*67,4*	*92,3*	*6,5*
	Average	***34,5***	***33,6***	***42,0***	***62,7***	***83,9***	***3,8***

The next step will be the identification of 3-4 best genotypes based off on farm performance and fodder quality.

Results for Soudanian Zone
In Soudanian zone trials were conducted at Gampela research station. Data from 2015 and 2016 allowed the identification of 15 accessions with high yield in grain and fodder. Grain and fodder quality analysis were perform on those selected accessions (Tables 6 and 7).

Table 6: Agronomic performance of selected accessions for Soudanian zone

Entry	Designation	Agronomic performance							
		FLO	HPM	LOE	DAE	GsE	HI	GY	STY
2	KalpelgaxPE03922	62	221	25	2,6	62	23	547	2474
3	PE0437xPE00515	66	196	36	2,4	70	42	911	2193
6	MISARI-2	69	210	34	2,7	65	28	771	2755
7	PE05578 C1	68	218	32	2,7	55	37	703	1917
10	Local Djibasso C0	64	214	38	2,4	63	54	990	1859
12	PE00967 C0	69	212	44	2,6	59	43	927	2141
22	Group6	59	243	46	2,3	55	23	646	2854
24	Local Kamboinse	64	216	23	1,9	64	25	594	2396
27	PE00437XPE00273	67	242	28	2,3	60	40	943	2406
34	PE05539	67	223	29	2,2	52	34	807	2380
45	Indiana 05	68	237	38	2,5	45	17	354	3026
46	Djiguifa	68	250	36	2,2	54	20	516	2542
47	CzMil Aristé	57	223	33	2,3	52	30	833	2797
48	NKO/TC1	68	219	33	2,4	61	36	729	2016
100	THIALACKZ	64	224	57	2,3	55	38	703	1823
	Minimum	*57*	*196*	*23*	*1,9*	*45*	*17*	*354*	*1823*
	Maximum	*69*	*250*	*57*	*2,7*	*70*	*54*	*990*	*3026*
	Average	*65*	*223*	*35*	*2,4*	*58*	*33*	*732*	*2372*

Table 7: Grain and Fodder quality of selected accessions for Soudanian zone

Entry	Designation	Grain characteristics		Fodder characteristics			
		Fer (mg/kg)	Zinc (mg/kg)	ADF	NDF	RFV	Protein
2	KalpelgaxPE03922	42	42	44,7	66,5	75,7	6,3
3	PE0437xPE00515	43	45	47,4	69,5	69,8	4,2
6	MISARI-2	34	37	51,0	72,9	62,8	5,1
7	PE05578 C1	37	41	52,0	74,9	60,1	3,3
10	Local Djibasso C0	38	39	50,5	75,1	61,5	4,2
12	PE00967 C0	37	36	53,2	76,9	57,8	2,7
22	Group6	35	39	51,9	75,1	60,2	3,9
24	Local kamboinse	34	34	50,7	73,1	63,1	3,8
27	PE00437XPE00273	34	39	51,1	74,1	61,9	3,1
34	PE05539	40	41	53,4	77,2	57,0	3,2
45	Indiana 05	39	36	48,7	70,1	67,8	5,2
46	Djiguifa	39	41	52,6	75,8	58,9	3,0
47	CzMil Aristé	34	35	51,3	75,1	60,8	3,6
48	NKO/TC1	43	37	52,0	74,7	60,3	3,4
100	THIALACKZ	37	37	48,9	71,5	66,2	5,4
	Minimum	*33,9*	*33,6*	*44,7*	*66,5*	*57,0*	*2,7*
	Maximum	*42,8*	*44,9*	*53,4*	*77,2*	*75,7*	*6,3*
	Average	*37,7*	*38,5*	*50,6*	*73,5*	*62,9*	*4,0*

The next step will be the identification of 3-4 best genotypes based on farm performance and fodder quality.

Niger

Following evaluation trials during the two years, data were analyzed using BMS package. The Genotype by Environment (G x E) interactions analysis resulted in 26 entries with grain yield superior to 2 tones/ha over the 2 agroecological zones. Fifteen varieties among this group were retained based on forage production for upcoming activities (Table 8). During the third year the selected germplasm was tested in on-farm environments in both the Sahelian and Soudanian regions on 4 and 2 farms, respectively. Six (6) entries (in yellow color) among the 15 were viewed as most promising by the rural participants during organized field visits. Their choice of test accessions was based on criteria such as adaptability to the growing area, early maturity, good tillering ability, plant height, thinness and number of fertile stems, length and spike's compactness, foliage density, and tolerance to lodging, and resistance to downy mildew attack.

Table 8: Characteristics of the selected entries

Entry	Designation	Origin	Fe*	Zn*	ADF	NDF	RFV	Protein	GrY	Stover	Daysfl	DMH
4	PE00397 x PE00515	Burkina	45,2	36,9	51,0	72,1	63,4	2,8	2208	6241	71	2,625
5	MISARI-1	Burkina	48,5	37,8	49,2	70,7	66,9	2,3	2242	7797	72	0,75
11	Sahel 22 C0	Burkina	38,8	32,2	51,5	75,1	60,8	2,4	2254	7283	69	0,75
12	PE00967 C0	Burkina	43,8	29,8	53,1	74,8	59,3	2,0	2014	8263	72	0,625
21	Group 4	Burkina	50,0	38,1	53,1	76,8	57,7	2,6	2241	6363	71	0,875
22	Group 6	Burkina	46,9	34,9	49,9	71,2	65,4	1,7	2339	6986	72	0,875
30	PE02987 X PE05347	Burkina	41,0	38,3	49,3	71,4	65,8	2,6	2041	7326	63	0,75
35	CzSyn 00-06	Mali	50,5	44,5	47,4	70,3	69,0	2,3	2297	6834	71	0,75
36	SoSat	Mali	41,5	37,3	48,9	72,7	65,1	3,1	2258	6512	62	1
39	CzSyn 00-02	Mali	44,1	33,3	50,7	74,1	62,0	2,0	2324	6292	74	0,625
43	Toroniou C1	Mali	45,7	38,6	51,5	74,1	61,2	2,6	2220	6704	70	1,125
50	Cz Syn 03 11	Mali	43,8	38,9	47,0	70,9	68,7	2,6	2077	6784	70	0,875
60	PE05913	Niger	45,5	35,7	50,8	75,7	60,9	2,7	2203	6112	71	1,875
61	PE00626	Niger	40,1	32,2	47,0	71,0	69,0	3,0	2077	7108	72	1,625
67	PE00437	Niger	61,1	44,7	52,9	75,4	58,9	2,5	2211	8144	78	0,5

*** in mg/kg**

Currently we are conducting for a second round a participatory varietal selection to identify the two or three accessions to be involved in future program. Food processing and nutritional quality tests will enhance this phenotypic selection.

5 – ACCOMPLISHMENTS

1. Regarding the objective of gathering the regional genetic material, we succeeded to gather 100 accessions from the 5 institutions that are INERA, INRAN, IER, ISRA and ICRISAT (see table 1 at the back). We can say that this objective has been realized at 100%.

2. Regarding the specific objective of assessing relationships between stover nutritional quality and digestibility and other agro-morphological traits, the grain quality of the different accessions have been sent to Niger at ICRISAT Sahelian Center for iron and zinc content. The results assisted in the selection of the 15 best entrees for each agroecological zone. The fodder was sent to Kansas State University for analyses of ADF, NDF and protein content. With the help of ICRISAT and KSU, this objective has been done at 100%.

3. Regarding the objective of selecting the 15 best entrees for each agroecological zone in each country, we tested all the 100 accessions in the two agroecological zones in all the four countries. In Senegal, the trial was conducted at Bambey and Nioro during 2016 raining season. In Mali, the trails were conducted at Cinzana and N'tarla during the raining season 2016. In Burkina Faso, the trials were conducted at Dori and Gampela during the raining season 2016 and in Niger, the trials were planted at Maradi and Bengou during the raining season 2016. A monitoring trip visited most all the trials except Maradi and

Bengou where Dr Zangré, the ex-PI have been mandated to visit. This objective has also been achieved.

4. The objective of identifying 3 to 5 farmer-preferred accessions for future promotion and use in pearl millet dual-purpose variety development has also been successful. At the end of this second year of on-farm trials, 15 entrees were tested and we will be able to select with participatory breeding system, to identify 3 to 5 good varieties for certification and cultivation at an on-farm field level in each agro ecological zone. The farmers must have a panel of diversified varieties where the most adapted one according to the agro climatic conditions can be selected. We will dispose of a group of early, medium and long duration varieties and according to the meteorological previsions and choose the most adapted variety to sow. This objective is done at 75% and will be completed at the end of this 2018 rainy season.

5. Farmers and producers have seen the importance of dual-purpose pearl millet varieties and a longer-term strategy for breeding highly nutritious dual-purpose pearl millet cultivars in West Africa is required. Getting at the same time food for the family, and fodder for animals, is a new slogan on the farm so we (as breeders) must try to satisfy this new demand. The genetic material we gathered in the first year constitutes an important collection for utilization in breeding for dual purpose varieties. This collection is very rich in terms of variety development. We have materials with early, medium and late maturing accessions, different colors and size of grains, short, medium and high variety types, short medium and long panicle types. In conclusion, with this collection we can select varieties for direct cultivation and genetic materials adapted to West and Central African climate and agroecological zones for varietal creations. The objective to contribute to the long-term breeding strategy was successful in the first year of the project.

6 - UTILIZATION OF RESEARCH OUTPUTS

As mentioned above, the results for this project has changed the research strategy in West Africa.

1. This onfarm demonstration plots conducted during 2017 and 2018 raining seasons were very important. Farmers, producers, extension service and governmental officers who have participated in the different field days organized and have really appreciated the varieties and they expressed their needs for utilizing the varieties.

2. Farmers, producers and policymakers understand the importance of dual-purpose millet varieties.

3. As livestock is a part of farmer livelihoods, food for the animals has been and will be always a priority for the farmers. We have been informed that some farmers used to sell important goods such as sheep, family jewels, etc. to buy food for animals when the rainfall failed.

4. These demonstration plots and dissemination methods must be continued over a broader area.

5. Feeding human and animals is a preoccupation in West Africa due to climate change.

ANNEX

Table 1: Entry list and original countries

Entrée	Pedigree	Type variétal	Code	Bloc	RI	RII
1	PE05887	Var exp (OPV)	SMILBF1	1	411019	412140
2	KalpelgaxPE03922	Var exp (OPV)	SMILBF2	1	411056	412101
3	PE0437xPE00515	Var exp (OPV)	SMILBF3	1	411061	412104
4	PE00397xPE00515	Var exp (OPV)	SMILBF4	1	411084	412116
5	MISARI-1	OPV	SMILBF5	1	411003	412141
6	MISARI-2	OPV	SMILBF6	1	411006	412108
7	PE05578 C1	Var exp (OPV)	SMILBF7	1	411005	412128
8	PE08030 C0	Composite C0	SMILBF8	1	411043	412127
9	PE08011 C0	Composite C0	SMILBF9	1	411076	412200
10	Local Djibasso C0	Composite C0	SMILBF10	1	411021	412196
11	Sahel 22 C0	Composite C0	SMILBF11	2	411032	412145
12	PE00967 C0	Composite C0	SMILBF12	2	411071	412177
13	IKMP 1	OPV	SMILBF13	2	411081	412117
14	IKMP 5	OPV	SMILBF14	2	411044	412103
15	IKMV8201	OPV	SMILBF15	2	411039	412134
16	PE01203 X PE05980-R3	Var exp (OPV)	SMILBF16	2	411098	412156
17	PE02987 X ICMV IS 92222	Var exp (OPV)	SMILBF17	2	411065	412175
18	PE02853xPE00404 R3	Var exp (OPV)	SMILBF18	2	411095	412150
19	Group1	Var exp (OPV)	SMILBF19	2	411079	412107
20	Group3	Var exp (OPV)	SMILBF20	2	411010	412191
21	Group4	Var exp (OPV)	SMILBF21	3	411082	412184
22	Group6	Var exp (OPV)	SMILBF22	3	411041	412105
23	Groupe7	Var exp (OPV)	SMILBF23	3	411070	412155
24	Local kamboinse	local	SMILBF24	3	411096	412168
25	Local Nahartenga	local	SMILBF25	3	411080	412172
26	GBxMORO	Var exp (OPV)	SMILBF26	3	411085	412187
27	PE00437XPE00273	Var exp (OPV)	SMILBF27	3	411036	412110
28	PE00437XICMVIS92222	Var exp (OPV)	SMILBF28	3	411064	412147
29	PE00397XPE00404	Var exp (OPV)	SMILBF29	3	411052	412180

30	PE02987XPE05347	Var exp (OPV)	SMILBF30	3	411059	412188
31	PE053/73	Parent_R (OPV)	SMILBF31	4	411089	412195
32	IBMV8402	OPV	SMILBF32	4	411031	412198
33	ICMVIS89305	OPV	SMILBF33	4	411086	412154
34	PE05539	Var exp (OPV)	SMILBF34	4	411062	412164
35	CzSyn 00-06		SMILML1	4	411017	412182
36	SoSat		SMILML2	4	411088	412112
37	CzSyn 00-01		SMILML3	4	411094	412115
38	CzSyn 03-10		SMILML4	4	411060	412129
39	CzSyn 00-02		SMILML5	4	411093	412111
40	Cr Toroniou HTC	Hybrid	SMILML6	4	411090	412146
41	CzToroniou HTC Aristé	Hybrid	SMILML7	5	411023	412161
42	Cz Maïwa HTC aristé	Hybrid	SMILML8	5	411074	412142
43	Toroniou C1		SMILML9	5	411027	412193
44	Sanioba 03		SMILML10	5	411009	412186
45	Indiana 05		SMILML11	5	411018	412124
46	Djiguifa		SMILML12	5	411042	412137
47	CzMil Aristé		SMILML13	5	411022	412109
48	NKO/TC1		SMILML14	5	411072	412199
49	Boboni		SMILML15	5	411001	412159
50	Cz Syn 03 11		SMILML16	5	411012	412169
51	Civarex/trombédié/guéfoué 16		SMILML17	6	411063	412181
52	PEO6001		SMILML18	6	411053	412139
53	PE02983		SMILML19	6	411047	412192
54	PE05607		SMILML20	6	411035	412190
55	IP8688		SMILNG1	6	411069	412122
56	PE02945		SMILNG2	6	411028	412149
57	B9-Tabi		SMILNG3	6	411020	412143
58	PE02831		SMILNG4	6	411013	412130
59	PE00040		SMILNG5	6	411030	412123
60	PE05913		SMILNG6	6	411046	412148
61	PE00626		SMILNG7	7	411002	412118

62	PE05346		SMILNG8	7	411068	412160
63	PE01491		SMILNG9	7	411092	412194
64	PE02898		SMILNG10	7	411040	412157
65	PE02603		SMILNG11	7	411055	412153
66	PE05387		SMILNG12	7	411066	412131
67	PE00437		SMILNG13	7	411038	412165
68	PE00456		SMILNG14	7	411057	412135
69	PE02983		SMILNG15	7	411015	412176
70	PE08039		SMILNG16	7	411014	412174
71	HK-MORO		SMILNG17	8	411025	412163
72	GAMOGI		SMILNG18	8	411049	412125
73	GA-HKP		SMILNG19	8	411011	412136
74	BOUDOUMA		SMILNG20	8	411029	412179
75	TTCHININ-BIJINI		SMILNG21	8	411083	412183
76	HKB		SMILNG22	8	411067	412158
77	H80-10GR		SMILNG23	8	411026	412173
78	SL-516	Landrace	SMILSG1	8	411077	412151
79	SL-49	Landrace	SMILSG2	8	411034	412102
80	SL-56	Landrace	SMILSG3	8	411004	412126
81	SL-69	Landrace	SMILSG4	9	411045	412152
82	SL-101	Landrace	SMILSG5	9	411097	412114
83	SL-328	Landrace	SMILSG6	9	411091	412119
84	SL-167	Landrace	SMILSG7	9	411024	412185
85	SL-170	Landrace	SMILSG8	9	411048	412162
86	SL-214	Landrace	SMILSG9	9	411033	412138
87	SL-215	Landrace	SMILSG10	9	411007	412120
88	SL-416	Landrace	SMILSG11	9	411016	412144
89	SL-523	Landrace	SMILSG12	9	411050	412132
90	SL-510	Landrace	SMILSG13	9	411099	412133
91	SL-515	Landrace	SMILSG14	10	411073	412121
92	SL-106	Landrace	SMILSG15	10	411054	412166
93	SL-45	Landrace	SMILSG16	10	411037	412171
94	SL-232	Landrace	SMILSG17	10	411008	412189

95	SL-526	Landrace	SMILSG18	10	411078	412113
96	SL-323	Landrace	SMILSG19	10	411058	412106
97	SL-442	Landrace	SMILSG20	10	411051	412178
98	SL-453	Landrace	SMILSG21	10	411087	412197
99	SL-418	Landrace	SMILSG22	10	411100	412167
100	THIALACK2	Var exp (OPV)	SMILSG23	10	411075	412170

ASSESSMENT OF PRODUCTION PROBLEMS IN WEST AFRICA AND MOLECULAR DIVERSITY OF PEARL MILLET PARENTAL LINES

1 – PRINCIPAL INVESTIGATOR

Desalegn D. Serba - *Kansas State University, USA*

2 – RESEARCH TEAM

Co-Investigators: Roger Zangre - *INERA/CREAF, Burkina Faso*
Ousmane SY - *ISRA, Senegal*
Moussa Daouda SANOGO - *IER, Mali*
Amadou ISSAKA - *INRAN, Niger*

3 - PROJECT GOALS AND OBJECTIVES

The overall goal of this project was to assess pearl millet production constraints in West Africa SMIL target countries at post flowering stage to identify biotic and abiotic constraints for priority research intervention. It was also aimed at evaluating the performance of breeding materials and experimental hybrids developed in US at two locations in the region (Bengou, Niger and Bambey, Senegal). The second part was intended to document molecular diversity of inbred parental lines and germplasm accessions obtained from various sources and at KSU disposal. This was initiated to obtain genomic diversity information for preliminary heterotic grouping of the breeding population. The specific objectives that were achieved in this project were,

1. Assess pearl millet biotic and abiotic production constraints in Niger, Burkina Faso, Mali and Senegal and research challenges and opportunities.

2. Evaluation of OPV, hybrids, and inbred lines for yield potential and stress tolerance in two representative locations of Sahelian zone

3. Assessment of genomic diversity in pearl millet germplasm and breeding materials

4 - OVERVIEW OF ACTIVITIES

1. To achieve the goal of identifying the priority biotic and abiotic production constraints put forward a field visit was conducted from September 28 - October 09, 2016. The following research centers/stations and surrounding farmers' fields were observed per country.

    - Niger: Kollo Research Center and Ndounga station
    - Burkina Faso: Katchari, Dori, Gampela and Kamboise research stations
    - Mali: Cinzana and Ntarla
    - Senegal: Nioro du Rip and Bambey (Desalegn only)

    Participants involved in the monitoring visit were:

    - Desalegn D Serba, Millet breeder, Kansas State University (KSU)
    - Roger Zangre, Millet breeder, Burkina Faso and PI for Dual-purpose millet project
    - Issaka Ahmadou, Millet breeder, Niger

- Moussa D Sanogo, Millet breeder, Mali
- Ousmane Sy, Millet breeder, Senegal, and
- Nathanael Bascom, Assistant Director, Sorghum and Millet Innovation Lab (SMIL)

2. The evaluation of OPV, hybrids, and inbred lines for yield potential and stress tolerance was conducted in 2016 with a total of 100 entries at Bengou, Niger and Bambey, Senegal. The category and number of entries are as in the Table 1 below.

Table 1. List of materials tested in preliminary evaluation of pearl millet OPV, hybrids, and inbred lines for yield potential and stress tolerance in Bengou, Niger and Bambey, Senegal.

Entries	**Number of entries**
OPV/Accessions	41
Single cross hybrids	29
Top-cross hybrids	22
R-lines	8
Total	**100**

3. Assessment of genomic diversity using next-generation sequencing (NGS) technology platform called genotyping-by-sequencing (GBS) was conducted on 400 entries comprising germplasm accessions and inbred parental lines. The geographic origin/source of the materials as in Table 2 below. Approximately 70 to 100 mg fresh leaf tissue was collected from 2-4 plants per entry after 15 days of emergence, freeze dried and ground to powder. Genomic DNA was extracted was with the BioSprint 96 DNA Plant Kit. DNA sample was quantified using the PicoGreen dsDNA quantitation assay, then double digested with two restriction enzymes, PstI (5′-CTGCA/G-3′) and MspI (5′-C/CGG-3′). GBS libraries were constructed. The libraries were sequenced on Ion Torrent sequencing machine. The sequence reads were quality checked, trimmed, mapped to the draft genome sequence using and SNP markers calls performed using TASSEL 5.0. The markers were quality checked for missing data, minimum allele frequency, and InDels filtered out. The quality SNPs data were used for population structure analysis, diversity, linkage disequilibrium, and selection signatures per subpopulation identified.

Table 2. Pearl millet inbred lines and germplasm accessions studied for genomic diversity

Region	Country	Seed source (entries)	Genetic category
East Africa (EA)	Somalia Kenya Sudan	PGRC (3) GRIN (1) PGRC (6)	Germplasm accessions Germplasm accession Germplasm accessions
India	India	GRIN (16) UGA (26)	Inbred lines Inbred lines
Middle East (ME)	Yemen	PGRC(10) GRIN(14)	Germplasm accessions Germplasm accessions
Southern Africa (SA)	DR Congo Malawi Zambia Zimbabwe	GRIN (5) PGRC (12) PGRC (1) GRIN (12),PGRC (2)	Germplasm accessions Germplasm accessions Germplasm accessions Germplasm accessions
USA	Kansas Georgia Nebraska	ARCH (155) Tifton (27) UNL (8)	Inbred lines Inbred lines Inbred lines
West Africa (WA)	Algeria Burkina Faso Chad Gambia Mali Niger Nigeria	PGRC (9) PGRC (22),GRIN (1) PGRC (5) PGRC (2) GRIN (3) PGRC (50) PGRC (11)	Germplasm accessions Germplasm accessions Germplasm accessions Germplasm accessions Germplasm accessions Germplasm accessions Germplasm accessions

5 – ACCOMPLISHMENTS

A. Achievements by project objectives stated in the proposal

1. Shortage of rainfall, erratic distribution and high temperature are limiting to the productivity of the most adapted crop, pearl millet in the region. Downy mildew (Sclemspom graminicola (Sacc.) J. Schrlit) is the most troubling problem on pearl millet production in the region and needs a concentrated research effort in germplasm screening and study of the genetics of resistance. Identified Cinzana, Mali and Bambey, Senegal are the hot spot for downy mildew screening under natural infestation.

2. The accessions, hybrids, and inbred lines evaluated at two representative locations in the Sahelian zone several high yielding hybrids, accessions with good level of downy mildew resistance were identified. The result is summarized in Table 3 below.

Table 3. Days to flowering, grain yield (kg ha-1) and downy mildew scores (0-10 scale, 0=immune/highly resistant, 9=most susceptible) for entries tested in Bengou and Bambey, 2016

Location	**Days to flowering (days ranges)**			**Grain yield range (kg ha-1)**				**Downy mildew scores (0-10 scale)**		
	≤40	41-60	≥61	≤500	501-1000	1001-1500	≥1501	0-3	4-6	7-10
Bengou	0	64	34	76	11	1	0	32	33	33
Bambey	14	58	5	32	22	10	7			

3. A total of 400 samples were sequenced three times on Ion Proton. More than 540 million unique reads were obtained. A total of 103,186,800 markers were identified using the TASSEL5-reference pipeline against the new pearl millet reference genome. After filtering the SNPs for 20% missing, 1% MAF, and InDels 82,112 genome-wide SNPs markers discovered. Approximate average marker density of 48.3 per Mb of the genome was calculated. Population structure analysis identified six sub-populations that mostly overlap with the genetic origin of the germplasm accessions/source of inbred lines. The principal component analysis showed the population structure and within subpopulation diversity as PCA1 and PCA2 explained less than 10% of the genetic diversity of the population (Figure 1). A neighbor-joining phylogeny analysis grouped the materials into 12 sub-clusters with West Africa as a base. The most clusters were formed among the inbred lines developed in USA. Genome-wide linkage disequilibrium analysis found the faster LD decay in West African sub-population than the rest of the sub-populations. Assessment of genome-wide patterns of nucleotide variation within each sub-population revealed that average genome-wide FST is higher in the Middle East followed by east Africa. The differentiation within Indian sub-population was low.

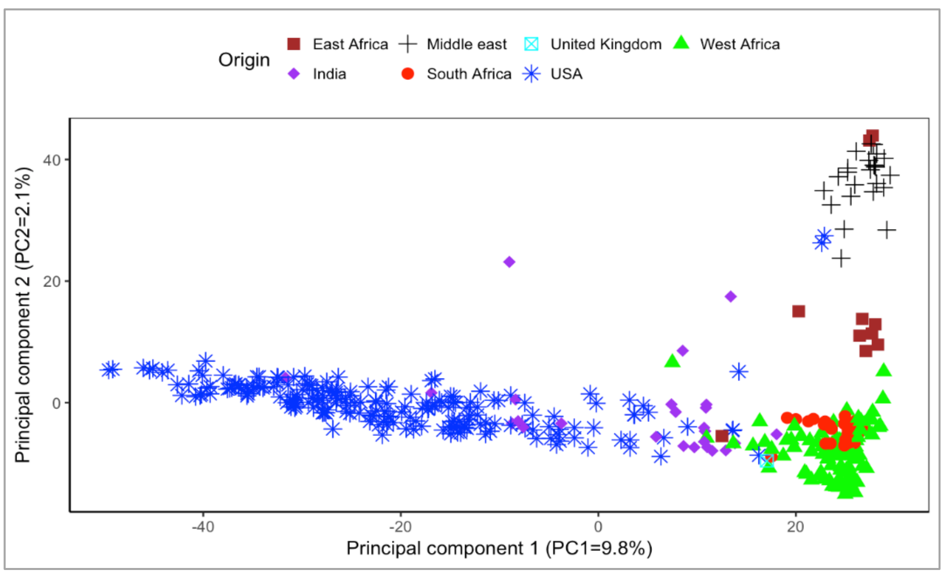

Figure 1. Principal component of 398 entries of germplasm accessions and inbred lines using 82,112 SNP marker data

B. Major challenges encountered and resulting project adjustments.

No major challenges were encountered that force to modify the objectives.

C. Student training achievement

The project has no training and outreach component.

D. Short-term training and outreach

The project has no training and outreach component.

6 - UTILIZATION OF RESEARCH OUTPUTS

The outcome of the pearl millet production constraints assessment and preliminary evaluation are exploratory by nature and serve as basis for future research activity to address the constraints identified.

Genomic diversity analysis was to identify heterotic groupings and also to detect quantitative trait loci (QTL) for important traits. Crossing within and between clusters will be conducted to substantiate the heterotic grouping. Field phenotyping will be conducted to conduct marker-trait association and detect QTL for different traits. The marker linked to the QTLs will be used in marker-assisted selection in the future.

Publications

Two review articles and a proceeding were published. A book chapter was submitted for publication. A manuscript on genomic diversity is in internal review for submission to the journal of The Plant Genome.

Peer reviewed journal articles

Desalegn D. Serba and Rattan S. Yadav. 2016. Genomic tools in pearl millet breeding for drought tolerance: Status and prospects. Frontiers in Plant Science. doi: 0.3389/fpls.2016. 01724.

Desalegn D. Serba, Ramasamy Perumal, Tesfaye T. Tesso, and Doohong Min. 2017. Status of global pearl millet breeding programs and the way forward. Crop Sci. 57:2891–2905.

Book chapter

Desalegn D. Serba, Rattan S. Yadav, Rajeev K. Varshney, S. K. Gupta, M. Govindaraj, Rakesh Srivastava, Rajeev Gupta, Ramasamy Perumal, and Tesfaye T. Tesso. Pearl millet: A resilient crop for arid and semi-arid environments. Chittaranjan Kole (editor). DESIGNING OF CLIMATE-SMART CROPS (Submitted)

Proceedings and Research reports

Desalegn D. Serba. Genetic improvement of pearl millet in USA: Past and present. 2018. Proceedings of International Millet Symposium: Finding New Markets and Uses for Millet. August 8-11, 2018 Fort Collins, CO, USA (In proceedings).

D. D. Serba and A. Obour. 2017. Nitrogen and phosphorus application effects on pearl millet forage yield and nutritive value. Kansas Agricultural Experiment Station Research Reports Vol. 3: Issue 3. doi.org/10.4148/2378-5977.1392 (Research report)

Manuscript in review

Desalegn D. Serba, et al. Genetic diversity, population structure, and linkage disequilibrium of pearl millet inbred lines and germplasm accessions

Presentations

Desalegn D. Serba. Pearl Millet Breeding Program at Kansas State University. 2016 SMIL Annual Research Review Meeting, March 7-10, Saly, Senegal. Feed the Future Innovation Lab for Collaborative Research on Sorghum and Millet, 2016 Annual Project Review Meeting, March 7-10, Saly, Senegal (Oral presentation)

Roger G. Zangre, Ousmane Sy, Moussa D. Sanogo, Issaka Ahmadou, and Desalegn D. Serba. Performance of Selected Pearl Millet Genotypes for Grain and Fodder Yields in West Africa. ASA-CSSA-SSSA 2016 Annual Meeting, Nov. 6-9, Phoenix, AZ (Poster).

Desalegn D. Serba. Genetic Improvement of Pearl Millet in USA: Past and present. International Millet Symposium: Finding New Markets and Uses for Millet. August 8-11, 2018 Fort Collins, CO, USA (Oral resentation).

Desalegn D. Serba. Kansas State University Pearl Millet Breeding Program. Feed the Future Innovation Lab for Collaborative Research on Sorghum and Millet 2018 West Africa Regional Pearl Millet Convening September 4-6, 2018 (Oral presentation).

Desalegn D. Serba. Genetic diversity, population structure, and linkage disequilibrium of pearl millet. 2018 ASA-CSSA Annual Meeting (Enhancing Productivity in a Changing Climate), Nov. 4-7, Baltimore, MD (Accepted for oral presentation)

7 - STATEMENT ON PROPOSED FUTURE ACTIVITIES FOLLOWING PHASE I RESEARCH ACCOMPLISHMENTS

A. Statement on future activities in phase II

The extent of the pearl millet production constraints identified in the first phase calls for much integrated and comprehensive research on priority researchable areas in pearl millet value chain. Drought, heat, low soil fertility are the major abiotic factors limiting yield of pearl millet. Among diseases, downy mildew is the most devastating in almost all areas. Smut disease, and parasitic weed striga are also inflicting significant yield losses. The farmers are largely growing landraces which are relatively resistant to the diseases but very low in yield potential. These situation kept the productivity of the crop very low and its economic benefit to the farmers is limited. Food security is still a big challenge in the region where the crop is the main staple food.

Firsthand information indicates that there are seed growers with good level of capacity to produce the hybrid seed with minimum technical top up from the researchers and development agents. Local company and cooperatives involved in processing of pearl millet grain are mushrooming in the region with significant market acceptance. These two important value chain needs to be supplied with the necessary input from the research system to play their role in the system efficiently. To do so the research system needs to play the pivotal role in developing parental lines to obtain varieties with required quality standard sought by the processors.

There have been many players in the region that mostly focused on a specific areas with little impact potential. As a result, there has not been any tangible yield improvement of pearl millet in the region. In some of the countries, there not even varieties released since 1994. This has to change with farmers' participation in the development of a real yield improvement options that are acceptable in the domain of their socio-economic conditions.

Therefore, the second phase of SMIL need to focus on target traits such as grain yield genetic potential, disease resistance, abiotic stress adaptation, and nutritional quality in an integrated and systematically designed research in the region. Pearl millet is known to exhibit hybrid vigor reaching 30% of the inbred parental lines and hybrid breeding in India has brought remarkable yield improvement of the crop. However, farmers in west Africa in general are still growing the open pollinated varieties and the yield is stagnated or even worsened with soil degradation and climate change.

It is my contention that regionally coordinated hybrid breeding with the available parental lines in the region and elsewhere will bring significant grain yield improvement. The national programs are working on the parental lines development but limited in capacity. Integrating the hybrid breeding initiative of the national programs across the region including from ICRISAT Sahel Center (ISC) will boost the capacity. Crossing among the seed parents and pollen parents from different counties in the region is may exhibit a better level of heterosis (hybrid vigor) than parental lines developed in a country. This approach can be implemented by conducting crosses at one station per country and evaluation of the hybrids at eight locations in the region (two per country). In the selection of the test locations the Sahel and Sudan zone will be represented systematically. Plant morphology, phenology, disease resistance, water use efficiency in comparison with local cultivars and parental lines will be recorded. To sustain the hybrid breeding, evaluation of populations and germplasm enhancement should complement the hybrid breeding approach.

In addition to food security, nutritional security is also challenging in the region. Micronutrient density and protein content of the varieties grown is not known much. Pearl millet biofortification for micronutrient density mainly iron (Fe) and zinc (Zn) has become practical. Enhancement of these micronutrients is highly important in the region where the general population cannot supplement their diet with other food sources. Characterization of breeding populations, OPV in pipeline, landraces and commercial cultivars for protein, Fe and Zn content needs to be conducted. It is also important to assess the phytate content that impede the accessibility of these micronutrients for human body. The effect of genotype-by-environment interaction (GxE) on the density and accessibility the nutrients needs to studied. To improve the nutritional quality of the hybrid varieties to be developed in the region research particularly using adaptable restorer lines developed at ICRISAT with enhanced Fe and Zn content needs to be utilized. This research area can be conducted in collaboration with the ICRISAT scientists in the region. In addition to micronutrient density other quality traits important for processing such as couscous, masa, etc., need to be assessed in collaboration with local processors in the region.

Comprehensive germplasm screening for disease resistance mainly downy mildew in hot spot sites and identification of sources of resistance is the priority research need to curb the problem. With this study of the genetics of resistance and identification of candidate genes for resistance needs to be known. Germplasm accessions from national gene banks, lines and populations from breeding programs, ICRISAT core collections needs to be assembled and screened for resistance in hot spot locations. In addition to field condition, screening under controlled conditions using the procedure developed at ICRISAT should be conducted. This is one area of research trainees may conduct. Application of NGS made candidate gene identification and gene transfer across genotypes very efficient in a reasonably short period. Therefore, the screening needs to be complemented with NGS of choice and data analysis. Then, the most resistant ones will be tested for restorer/maintainer type. Then conversion to the respective inbred parental lines will be proceeded.

Drought and heat are the major environmental constraints especially in the Sahelian zone where pearl millet is mostly the only cereal grown. Screening the germplasm for drought tolerance at seedling stage in controlled environment (growth chamber) and using pollen germination at different temperature gradients (35-40°C) as a proxy for heat tolerance will identify sensitive and tolerant genotypes. Moreover, characterization under replicated drought and heat stressed field conditions for physiological traits such as tillering capacity, photosynthetic efficiency, water retention, and recovery after prolonged drought will substantiate the finding of the controlled environment screening. Furthermore, leaf rolling characteristics under drought and heat, wilting and senescence, relative water content retention, and osmotic potential will be studied to understand and the mechanism of tolerance. This will also be complemented with genome-wide association study for the traits to identify candidate genes for heat and drought tolerance. In this process an appropriate reduced representation genome sequencing platform will be conducted. Markers will be developed and marker-trait association will be conducted. From the pearl millet genome sequence and resequencing of many genotypes genes for wax accumulation on the leaf surface are underlying both drought and heat tolerance. Therefore, looking for genotypes with enriched alleles of the genes for wax accumulation in the genome using NGS will facilitate the improvement of the adaptation to these stresses.

B. Linkage to Phase I objectives and activities

The phase I breeding research on pearl millet was mostly exploratory. The K-State researcher came on board late, half way the first phase project implementation. With the resources available and time remaining in phase I necessary steps of identifying the burning problem, preliminary evaluation of hybrids as compared to OPV and parental lines, and genomic diversity of the breeding population was conducted. The dual-purpose pearl millet screening conducted three years and give a good overview the regional collaboration to implement more structured and thorough breeding research in the region. The above proposed are of research can be implemented in collaboration with mostly researchers trained in the phase I. A breeder was trained on downy mildew in Senegal, a breeder each were trained from Niger and Burkina Faso. These freshly graduated researchers need to engage in such structured and regionally coordinated research to further their practical knowledge.

C. Training and outreach objectives

Training human power for sustainable implementation of the research. To sustain the yield and nutritional quality research on pearl millet, man power training in these areas is a high priority. The available researcher in the region is below the critical mass as compared to the economic value of the crop. Efficient utilization of the available human resources in the region and additional training to fill the limited man power gap of the national programs should be carefully planned. The training also needs to encompass local actors, extension agents, seed producers, and farmers themselves with gender sensitivity. Women farmers, input and output traders, processors need to be counted in short training to deliver the utmost contribution and advance the productivity and the economic benefit of the crop to the society.

OPTIMIZATION OF THE SEEDBALL TECHNOLOGY FOR PEARL MILLET, AND AGRONOMIC AND SOCIO-ECONOMIC EVALUATION IN THE CONTEXT OF SMALLHOLDER FARMERS IN SENEGAL AND NIGER

1 - PRINCIPAL INVESTIGATOR

Ludger Herrmann – *University of Hohenheim, Germany*

2 – RESEARCH TEAM

In this project the University of Hohenheim as lead institution collaborated with partners in Niger and Senegal. The University of Hohenheim was responsible for project management and development of the seedball technology per se, e.g. in greenhouse and climate chamber trials and conducted scientific studies on the mechanisms behind seedball performance. The national agricultural research partner in Niger was INRAN (Institut National pour la Recherche Agronomic du Niger), CERRA Maradi, in personas Dr. Maman Nouri and Dr. Hannatou Moussa. In Senegal, Dr. Ousmane Sy from ISRA (Institut Senegalais de Recherches Agricoles), Bambey station took over responsibility. The NARS did project management at the national level, conducted on-station trials, and facilitated on-farm trials with farmer organizations. In Senegal, the participating farmer organization was FAPAL (Federation des Associations PAysans de la region de Louga). The respective partner in Niger was FUMA GASKIYA (Federation des Unions de producteurs de MAradi GASKIYA). The farmer organisations were responsible for on-farm trials, distribution of the technology, and development of local information materials (poster, radio and video spots). At a later stage, different education institutions of the partner countries supported thesis works of local students (Ecole Nationale Superieur d'Agriculture, Universite de Thies, Universite de Tahoua, Universite de Maradi).

3 - PROJECT GOALS AND OBJECTIVES

The Sahel is one of the harshest environments of the globe. The climate is characterized by high temperatures and a short rainy season and the soils are mostly sandy with low water holding capacity and chemically poor. As a result, people mainly rely on subsistence agriculture with a high risk of food shortage. The major staple crop is pearl millet. Due to the short rainy season (about 90 days) much of the final yield is depending on a successful early establishment of the crop, which is at risk due to an unreliable start and continuation of the rainy season.

Therefore, the ultimate goal was to develop a sowing technology that supports early plant vigor and crop establishment of the major staple crop pearl millet in order to increase yield and income and at the same time reduce cropping risk and loss on investment.

The seedball technology, that was developed in the framework of permaculture by Masanobu Fukuoka in the early seventies, appeared as a technology that could serve the defined goal, since it is simple, i.e. applicable by any farmer, and based on local resources, i.e. it implies a low economic risk. In addition, it allows to add amendments like nutrients or pesticides on demand.

The major project objectives were:

1. To optimize the seedball technology for pearl millet using locally available resources
2. To validate the performance under Sahelian field conditions

3. To strengthen the seedball research capacity in the partner countries, and

4. To take into account gender specific constraints in order to assure equitable participation.

4 - OVERVIEW OF ACTIVITIES

Approach

The activities were, with respect to participation, organized in a pyramidal structure with temporally overlapping activities. Technology development was in the hand of scientists with intermediate backstopping with local partners and farmers. Optimization was based on greenhouse and climate chamber experiments. The same is true for experiments dedicated to understand mechanisms behind seedball performance. Once optimized, seedballs were tested using local materials on-station. Showing sufficient germination on-station, on-farm mother (researcher managed, structured complete experiments with simple spatial organization) and baby plots (farmer managed, free choice of technology options but including local control) were initiated.

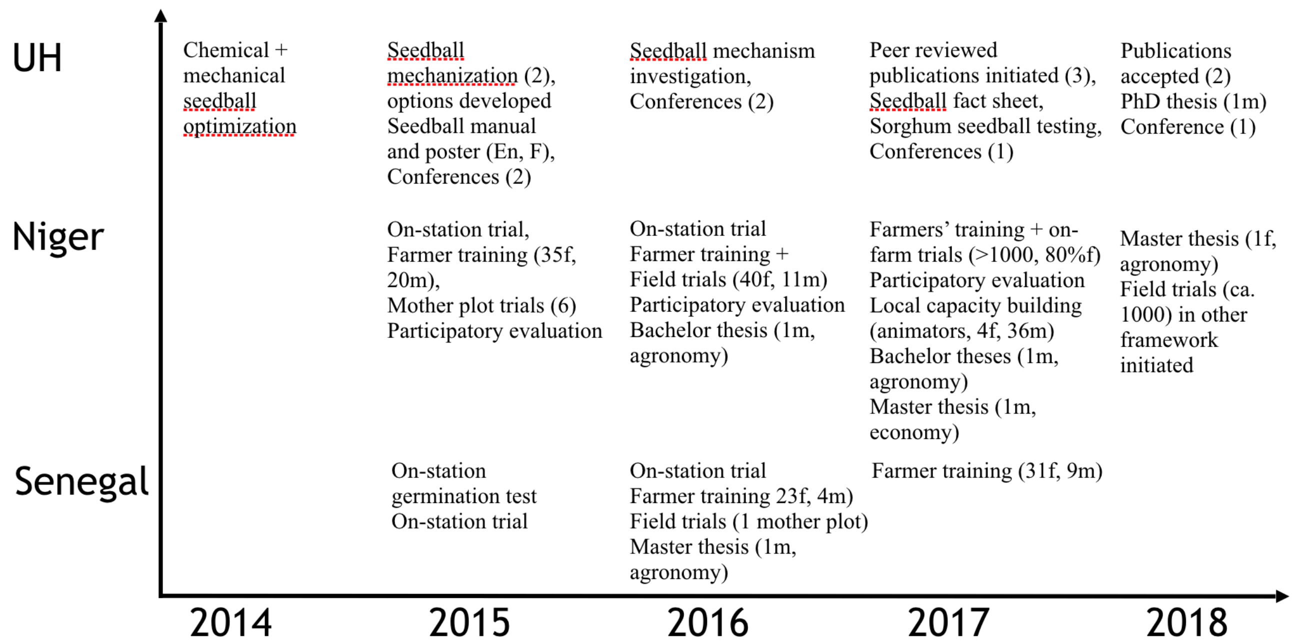

Figure 1: Overview of activities 2014 to 2018 in the seedball project

Temporal order (Figure 1)

The first year was dedicated to mechanical (compound mixtures, diameter) and chemical optimization (i.e. mainly nutrient additives) of the seedballs in greenhouse experiments. Once this was achieved, they were successfully pre-tested in the off-season in Senegal with local materials. Next step was the testing in-season and on-station in Niger and Senegal. In 2015, in Niger, already researcher managed mother trials in 6 villages were conducted. Parallel to agronomic on-station and on-farm trials, mechanization options were developed and tested during seedball production workshops, and experiments were conducted to unravel the mechanisms behind seedball success. While farmer participation in Niger steadily increased over time, the collaboration failed in Senegal. During the project time six students (1 Phd, 4 Master, 1 Bachelor) were trained. The topics did mostly originate from the field of agronomy. Seedball knowledge distribution occurred via reviewed publications

(3), oral and poster presentations (11), a manual (English, French), a poster (English, French, Haussa) a video (Haussa, French subtitles), and two radio spots (Haussa).

5 - ACCOMPLISHMENTS

Preliminary remark

The project was prepared by one agronomic master thesis (Jan Mühlena) and one agro-sociological bachelor thesis (Konny Biegert) conducted in Senegal. These showed that ex-ante there are neither economical or agronomic nor social or religious factors that would impede seedball adoption per se. Under the environmental conditions of Senegal sufficient germination rates could be achieved with pearl millet seedballs except if arabic gum was used as binder (Figure 2). Organic amendments did not improve pearl millet performance, probably due to the low amount of nutrients added. Therefore, wood ash and NPK as multi-compound fertilizer were chosen for further testing.

A. Achievements by project objectives

O1: To optimize the seedball technology for pearl millet using locally available resources.

1.1 - In a first step, the seedballs were mechanically and chemically optimized. It turned out that a diameter of 1.5-2.0 cm with 23-25 seeds stochastically distributed in the seedball were optimal for germination. With respect to chemical compounds, urea and ammonia compounds decreased germination rates (Figure 2). Therefore, if multi-compound fertilizer are used, they should not contain any ammonia. The amount of nutrient additives per seedball was maximized in order to support early establishment but not to decrease germination rates. The result is the following standard recipe: 50 g loam, 80 g sand, 2.3 g seeds, 25 ml water and either 3 g wood ash or 1 g NPK fertilizer (17/17/17).

Further increasing the amounts of nutrient additives would decrease germination via osmotic effects.

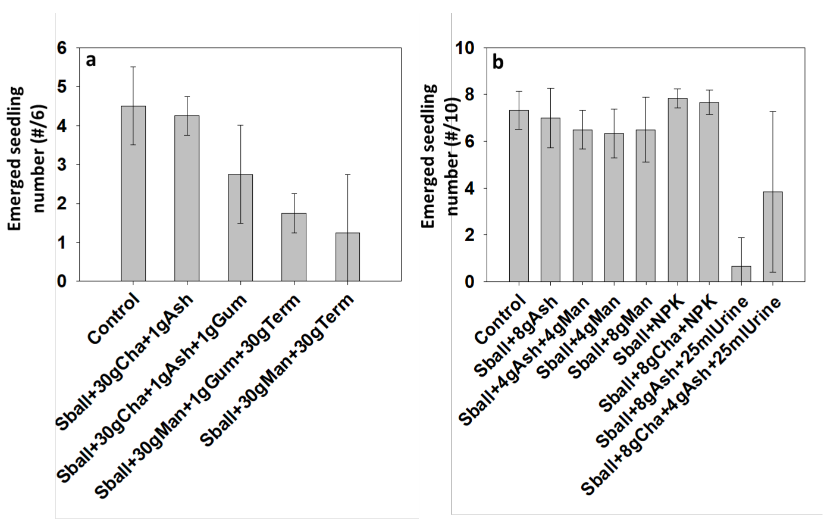

Figure 2: Treatment effects on pearl millet seedling number at the 7th day after sowing for (a) six and (b) ten seeds per seedball.
Bars represent arithmetic means (n=6) and error bars indicate standard deviations (±). Control = non-pelleted seeds, Sball = 80 g sand + 50 g loam + 25 ml water. Cha = charcoal, Ash = wood ash, Gum = gum arabic, Man = manure, Term = termite soil, NPK = 25 ml 17:17:17 mineral fertiliser in 200 ml g-1 solution and Urine = cattle urine.

1.1 - Climate chamber experiments with soil columns and i) computer tomographic evaluation of root development (Figure 3), ii) high spatial resolution soil water extraction, as well as iii) following chemical analysis revealed that seedballs enhance root proliferation in particular close to the seedball but also farther away. In effect, nutrients added to the seedballs are distributed to the surroundings by diffusion. This triggers root proliferation also outside the seedballs. This effect is short-lasting, only about two weeks. After these, the pearl millet plants have consumed the existing nutrient resources and additional fertilization has to start. Therefore, seedballs and fertilizer microdosing appear as complementary measures.

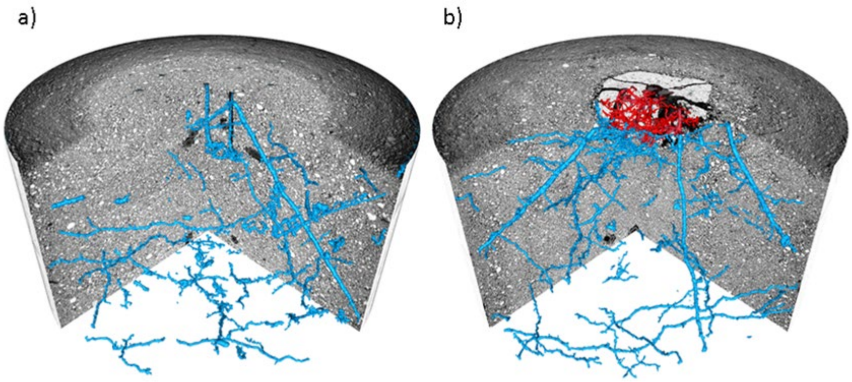

Figure 3: 3D-representation of the root system within the upper part of soil columns at day 21 after sowing: a) control treatment, b) seedball+NPK.

Roots within the seedball are colored in red, roots outside the seedball are colored in blue. The soil/seedball structure is presented in grey scale, with dark colors representing material with low electron density, i.e. air filled pores and light colors representing high electron density, i.e. quartz grains. Voxel side length is 40 µm, hence roots in the lowest diameter class (<200 µm) could only partially be presented.

1.2 - In an additional soil column experiment with irrigation it was shown that the water is preferentially directed via the disturbed zone caused by the sowing technique towards the seedballs. This effect might lead to lower water suction, that is higher water content around and in the seedballs. However, neither suction nor content could be measured in this experiment.

Table 1: Pearl millet panicle yield as affected by treatment interaction with soil type under dry and wet sowing in Maradi region of Niger Republic for the 2017 planting season.

Treatment	**Sball+1gNPK**		**Control**		**Sball+3gAsh**	
Factor	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**
Soil type (p=<.0001)						
Damba	3600 n=1	2023±339 n=15	2500 n=9	1287±386 n=38	1694±614 n=23	No data
Gueza	1051±639 n=1	1502±579 n=81	833±481 n=15	1155±536 n=153	938±311 n=8	1368±688 n=72
Jambali	1400 n=1	1651±692 n=22	1000 n=1	1297±533 n=35	No data	1690±533 n=13
Jigawa	828±395 n=21	1717±1013 n=300	684±408 n=38	1181±772 n=696	852±476 n=17	1492±1000 n=396
Guezami-guezami	No data	1280±565 n=6	No data	971±450 n=14	No data	993±215 n=8

Control = farmers' practice, Sball = 80 g sand + 50 g loam + 25 ml water, Ash = wood ash and NPK = 15:15:15 mineral fertilizer, n represents sample size.

Table 2: Pearl millet panicle yield as affected by treatment interaction with management as well as gender under dry and wet sowing in Maradi region of Niger Republic for 2017 planting season

Treatment Factor	Sball+1gNPK Dry	Sball+1gNPK Wet	Control Dry	Control Wet	Sball+3gAsh Dry	Sball+3gAsh Wet
Management (p=<.0001)						
Partial weeding	No data	2146±350 n=34	No data	1341±346 n=68	No data	2007±368 n=34
Full weeding	992±673 n=30	1637±935 n=390	764±487 n=55	1170±735 n=868	879±426 n= 25	1444±950 n=478
Gender (p=<.0001)						
Male	2100±2121 n=2	1917±1312 n=96	868±1139 n=4	1412±1091 n=206	141±16 n=2	1917±1451 n=110
Female	912±471 n=28	1607±746 n=328	755±421 n=51	1118±549 n=730	943±379 n=23	1362±686 n=402

Control = farmers' practice, Sball = 80 g sand + 50 g loam + 25 ml water, Ash = wood ash, NPK = 15:15:15 mineral fertilizer, n represents sample size.

In 2017 large-N on-farm trials have been conducted, where the farmers could choose between dry (i.e. before) and wet (i.e at the beginning of the rainy season) sowing. Due to the free farmers' choice the results are imbalanced. However, they show that the seedball technology enhances panicle yields in most cases, including wet and dry sowing. 2017 represents quite normal meteorological conditions. Therefore dry sowing was not widely practiced. Nevertheless, the available data show a yield increase with dry sowing when compared with the control. This is an indication that neither seed predation occurred nor that seedballs severely retarded crop establishment. The comparison between wet and dry sowing shows that wet sowing should be preferred under these "normal year" conditions. However, farmers told in post-season evaluations that under the drier conditions of 2016, seedballs performed better with dry sowing, and that seedball treatments gained yield whereas the control totally failed.

1.3 - Two low-cost mechanization options were developed. The idea was to keep investment costs as low as possible and to design options that can be fabricated and repaired by local craftsmen. The two options were tested by farmers but it turned out that they did not significantly increase the production efficiency, i.e. workload was not decreased. Meanwhile other mechanization options are available but need higher initial financial investment. For such a case, the project was financially not prepared and, consequently, could not invest in such an activity. But this remains a topic that could be approached in phase two. FUMA Gaskiya could then act as an intermediate, as it does for other group purchases like seeds or fertilizer, and develop a model how reimbursement and maintenance can be organized. FUMA Gaskiya sees in this endeavor a chance even for farmers to develop a business, by renting the machine and producing seedballs for other farmers.

1.4 - No problem was detected to produce performing seedballs with local materials at the testing sites in the West African Sahel. Sand, loam as binding agent, wood ash from kitchen stoves, and low amounts of NPK fertilizer were everywhere available. Slight differences in their chemical or grain size composition did not seem to play a major role.

1.5 - Participant numbers show that seedballs are a technology "naturally made" for women, who are not reluctant to invest work time and normally have low fertility fields far away from the villages. And for women dry sowing is attractive, since during the wet sowing time they need to help their husbands in their fields.

Farmers were keen to add more fertilizer compounds to the seedballs. However, this is not feasible due to osmotic effects reducing the germination rates. Farmers noted that sowing technique can be different on different local soil types. On very sandy soils (Jigawa), seedballs can be thrown onto the soil surface and introduced into the topsoil by simple trampling on them with the feet. On other local soil types (e.g. Gueza) this is not feasible and the normal sowing technique needs to be applied, i.e. opening the soil with a hand tool before putting the seedball. Men in particular asked for mechanization of the technology, also as a business option. In addition, it was requested to include fungicides and insecticides into the basic formula. The project could not respond to this in the first phase due to questions arising in connection to a respective EMMP. However, this could be tackled in phase 2.

O2: To validate the seedball performance under Sahelian field conditions and to determine the agronomic and socio-economic benefits for smallholder farmers in different contexts

2.1 - The large-N on-farm trial in Niger in 2017 revealed that in 856 out of 991 documented trials, the seedball variant achieved a higher yield than the control treatment, i.e. in 86% of the cases. In this trial of 10m*10m size 781 women (79%) and 210 men participated. Figure 4a shows that the seedball effect is not gender dependent, but that men achieve in tendency higher and the highest yields. This can be explained by the fact that they normally own the sites closer to the villages which are more often treated with any kind of organic residues.

The trials also show that the seedball effect is not restricted to one soil type, but that on all local soil types seedball application resulted mostly in positive yield effects and in a low number of failures (Figure 4b).

Figure 4c indicates that the combination of seedballs with other management innovations - in this case partial weeding - can lead to synergistic effects. The combination did not underperform in any case, and only one is lying on the 1to1 line.

Finally, a clustering effect can be noted, if results are evaluated taking the participating villages as variable. This is in some cases also true, for negative results produced by the seedball treatments. This effect is explained by Figure 5. It shows that in villages where experienced farmers conducted the trials, who were familiar with applying experimental protocols, the seedball treatments produce, in general, a positive yield effect. This was not the case in villages, where farmers contributed for the very first time to on-farm trials. In their cases, the treatment plots are very often sown later, and with respect to management neglected. This is due to the fact that they do not see the experimental plot as a proprietary one but more as a piece of land lent to the farmer federation.

In many cases, the NPK-seedball variant outperformed the wood-ash variant (Table 1). This might be explained by the N-component in the NPK fertilizer that is totally missing in wood ash. In some cases wood ash produces similar results. Wood ash seedballs are probably a suitable alternative for women, since they produce the ash in their kitchens and have less investment capital.

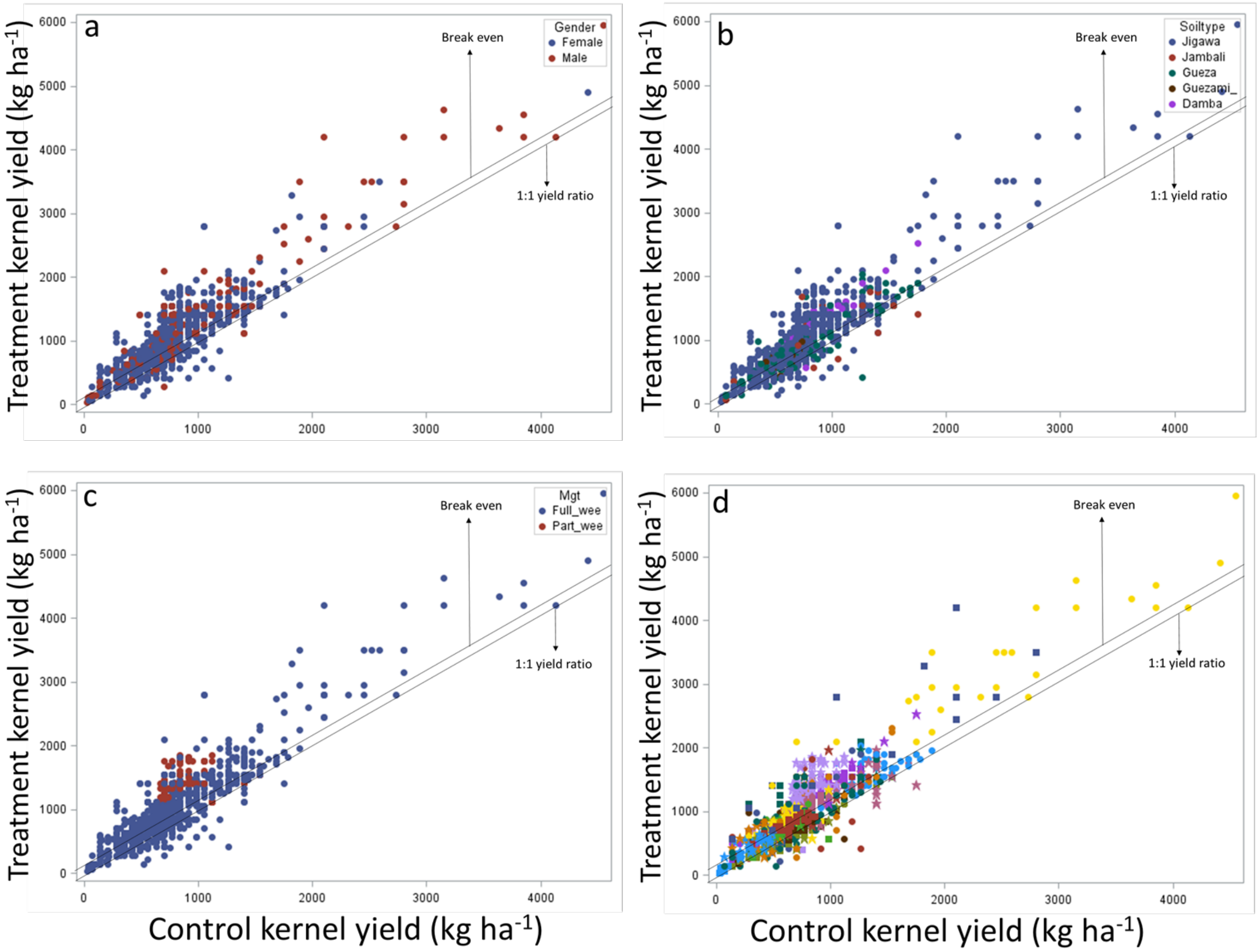

Figure 4: Agronomic performance of the seedball technology by comparison with control treatments in 991 on-farm trials in the Maradi region of Niger in 2017: a. gender aspect, b. soil aspect, c. management effect i.e. weeding, d. village effect

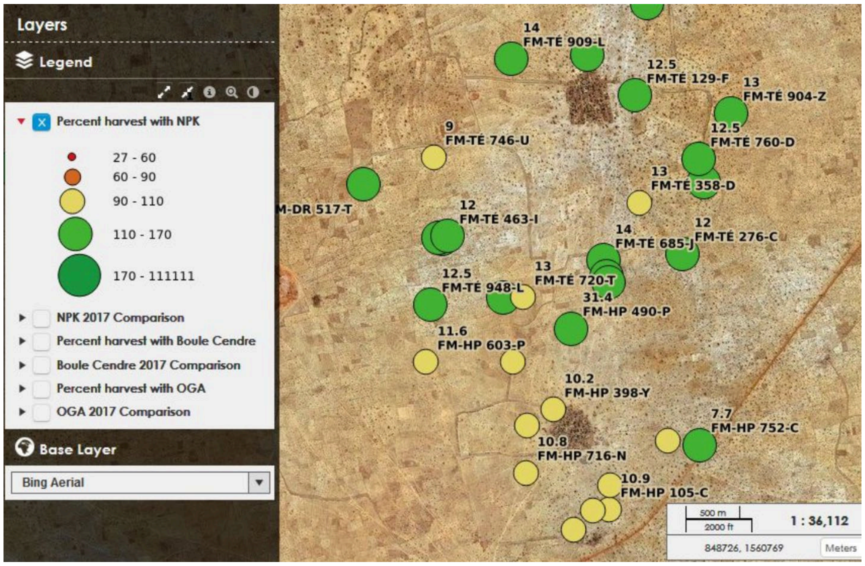

Figure 5: Effect of farmer experience on NPK-seedball on-farm field trials in 2017 in the Maradi region of Niger.
(Northerly village with farmers that have repeatedly participated in field trials under guidance of the farmer federation FUMA Gaskiya, southerly village with farmers who contributed to field trials for the very first time)

2.2 - During the project it was not possible to collect and analyze hard economic data, to date. One master thesis on this topic was begun in 2017 but never finalized due to strikes at Maradi University. However, from the farmer perspective, the seedball technology appears economically feasible otherwise not so many volunteers would participate in on-farm trials (>1000 in 2017 and 2018) and even non-members of the farmer federation would ask to be trained on it. In interviews in 2017 more than 95% of women and men farmers indicated that they are convinced by the technology, that they would continue to use it in the next season and that they would recommend it to other farmers.

The economic feasibility might be demonstrated by this simple calculation. For one ha pearl millet 10,000 seedballs are needed. Work load for production is about 40h. A daily labourer is hired at about 2500FCFA a day. Not considering costs for the locally available materials (mainly sand) production costs are 12500 FCFA. One kilogram of pearl millet costs about 200 FCFA (if bought as 100kg sack). In consequence, the point of economic break even is reached if about 65kg pearl millet seeds are additionally produced by this technology. In figure 4 this is visible by the majority of points lying above this production threshold line (control + 65kg). Additional points in favour of the technology are that i.) the additional biomass produced is not accounted for in this calculation, ii.) seedballs can be produced during the dry season when opportunity costs are low and even wages lower than 2500 FCFA, and iii.) seedballs allow for dry sowing which in turn reduces work load at peak demand that is during wet sowing at the beginning of the rainy season.

O3: To strengthen capacity for seedball research and application in Senegal and Niger

3.1 - A seedball production manual was produced by the PhD-student C. Nwankwo and translated into French by the Senegalese counterpart O. Sy. A translation into local languages did not appear reasonable, since most farmers in the intervention zone are illiterate. Therefore, a poster using pictograms was developed in English and translated to French and the Haussa language by the farmer federation FUMA Gaskiya, where most on-farm trials were conducted.

3.2 - The different stages of seedball development were presented repeatedly to an international audience at the Tropentag conferences 2015-2017. C. Nwankwo was awarded the best poster price among more than 1300 contributions in 2015.

3.3 - The farmer federation FUMA Gaskiya produced two radio spots in Haussa language and emitted it several times via the communitarian "Radio Binto" that covers the area north of Maradi. It also produced a video in Haussa with French subtitles and a poster for illiterate farmers. These materials were provided during an exchange visit to the farmer federation MOORIBEN working in southwest Niger. In Senegal, no radio spot was produced nor was the video translated due to lacking proof of performance on-farm.

3.4 - Three peer-reviewed journal articles were published or accepted for publication in 2018. These cover the pre-requisites for seedball application, the development of the technology itself, and the mechanisms behind its performance. A fourth one on the agronomic performance in on-farm trials is in preparation. During the course of the project intermediate results were presented as oral (5) or poster (6) presentations at different conferences, in particular the Tropentag and the annual conference of the German Soil Science Society.

In 2017 the farmer federation FUMA Gaskiya produced a video on the seedball production in Haussa language with French subtitles. Since meanwhile hundreds of videos on the seedball production are available on YouTube in different languages, a further translation was dismissed.

B. Major challenges encountered and resulting project adjustments

Three major challenges occurred in the course of the project.

1. On-station trials did not deliver reasonable results. This is due to over-fertilization of on-station fields. Therefore, data gathered on-station did not relate to results of on-farm testing. In consequence, on-station tests were skipped in 2017 and engagement redirected towards large-N on-farm testing.

2. The co-operation with the chosen Senegalese farmer organisation FAPAL was neither trustful nor efficient. In particular the gender question, i.e. participation of women, could hardly be addressed. In addition, there was not sufficient care taken with regard to the field trials. Therefore, in 2017 the co-operation was suspended and no further field trials planned or conducted.

3. It was difficult to recruit students and raise interest for this topic. In Senegal this was potentially due to the non-attractive compensation offered. In Niger, no university professors were per se part of the project and thus were not interested in offering the topic to their students. Therefore, a structural response needs to be formulated for phase 2 activities.

C. Student training achievements

A success is the defense of his PhD-thesis on the seedball technology by Charles Ikenna Nwankwo at the University of Hohenheim. Meanwhile he is an expert in this technology and ready to disseminate it to other pearl millet growing regions of West Africa.

The two master students in Senegal were trained by him on the seedball technology, but the institutional environment was not such that high level results were achieved.

In contrast, the bachelor and master students in Niger were well integrated in the project structure and received support from INRAN and FUMA Gaskiya, but their theses have not be defended due to failure of the educational system (student strikes, absence of supervisors).

D. Short-term training and outreach

Due to the good collaboration between FUMA Gaskiya and INRAN the short term training was a success story in Niger. Alone in 2017 more than 1000 farmers, about 80% being women, were trained. In addition, 40 animators (10% female) were introduced into the technology. Animators act in the structure of the farmer federation at village level as contact point and technical advisors of the farmer federation and control data collection.

By contrast, only about 70 farmers were trained at two locations in Senegal (2016 Louga, 2017 Bambey), but no reliable on-farm data were gained. Major reasons were i) low interest in pearl millet production (major crop is cowpea) of the originally participating farmer organisation (FAPAL), ii) great distance between NAR station and on-farm site, and iii) poor co-ordination. So on-farm activities in Senegal can be seen as a failure.

Outreach was achieved at different levels. Three peer reviewed journal papers are meanwhile published or accepted for publication. In addition, seedball technology was presented in five oral and six poster presentations during conferences in Niger, Senegal and Germany. Contacts were made with research institutes and private enterprises that are interested in the seedball technology in Canada, Pakistan and Germany.

6 - UTILIZATION OF RESEARCH OUTPUTS

A. Technologies and management practices by phase of development

The basic recipe for seedball production was detailed in a technology fact sheet in 2017.
Meanwhile more than 1000 on-farm tests were conducted in 2017 (evaluated) and 2018 (still under harvest, externally funded). In 2017, in Niger, from 991 evaluated on-farm tests, 856 seedball variants surpassed with respect to grain yield the control treatments; and 781 produced an additional kernel yield of more than 65kg, that is assumed to balance the production costs. This means that the seedball technology was agronomically successful in 85% of the cases and economically viable in 79% of the cases.

B. Intermediate outputs

Two mechanization tools were developed, but proved not to be efficient, since exclusively relying on handwork. Promising tools need to be motor driven in order to reduce workload. Several motor driven options have been presented meanwhile in the internet, but could not yet be tested or further developed.

C. Publications

Peer reviewed publications

Nwankwo and Herrmann (2018). Viability of the seedball technology to improve pearl millet seedlings establishment under Sahelian conditions - a review of pre-requisites and environmental conditions. IJAIR 6, 261-268

Nwankwo, C. I., Blaser, S. R. G. A., Vetterlein, D., Neumann, G. and Herrmann, L. (2018). Seedball-induced changes of root growth and physico-chemical properties – a case study with pearl millet. JPNSS 181/5, 768-776

Nwankwo, C. I., Mühlena, J., Biegert, K., Butzer, D., Sy, O., Neumann, G. and Herrmann, L. (2018). Physical and chemical optimisation of the seedball technology addressing pearl millet under Sahelian conditions. (Accepted with major revisions JARTS)

Theses

Cheikh Ahmed Tidiane Dieng (2016): Etude de l'effet de l'utilisation de la technologie boule de semences sur les performances du mil [Pennisetum glaucum (L.) R. Br.]. (Master thesis, Universite de Thies, Senegal)

Mouhamadou Moustapha Diome (2017): Etude socioéconomique des conditions de faisabilité et de transfert de la technologie boules de semence dans le département de Bambey. (Master thesis, ENSA, Senegal)

Iro Harou Ousseini (2017/18): Impact de technologie de boules de semences sur le rendement du mil en milieu paysan : Cas de la commune de Serkin Haoussa. (Bachelor thesis in preparation, Universite Dan Dicko Dankoulodo de Maradi, Niger)

Daouda Harouna Abassa (2017/18): Evaluation du taux d'adoption et rentabilité de la technologie de « boule de semence » sur la culture du mil (Pennisetum glaucum (L.) R. Br.) en milieu paysan : cas des communes de Djirataoua, Safo et Serkin Haoussa de la région de Maradi. (Master thesis in preparation, Universite Dan Dicko Dankoulodo de Maradi, Niger).

Charles Ikenna Nwankwo (2018): Development of seedball technology for smallholder pearl millet production in the West African Sahel. (PhD-thesis, University of Hohenheim, Germany)

Lena Geiger (2018/19): Relation between soil properties and performance of the seedball technology. (Master thesis in preparation, University of Hohenheim, Germany)

7 - STATEMENT ON PROPOSED FUTURE ACTIVITIES FOLLOWING PHASE I RESEARCH ACCOMPLISHMENTS

A. Statement on future activities in phase II

From the agronomic perspective, seedballs have shown to be efficient in certain Sahelian environments where subsistence agriculture is practiced mainly relying on handwork. For these further outreach should be envisaged. With respect to the technology itself, only a part of potential additives has been tested and higher impact might be reached if pesticides would be included in the basic formulation and the seedball technology applied in an integrated approach considering complementary innovations. First greenhouse trials indicated that the technology might be transferable to sorghum, too. With respect to gender, a greater share of men's involvement will probably only happen, if a mechanisation for seedball production is offered.

From the economic perspective, a real proof of viability could not be delivered in the first phase. Therefore, a new concept with new partners needs to be followed. In consequence, the following activities are proposed:

Activity 1: Phase 1 ex post survey of adoption rates in the Maradi region of Niger
Rationale: During phase 1 more than 2000 on-farm trials have been conducted. This great number already indicates that the seedball technology seems to be of interest for the local farmer organisation FUMA Gaskiya and its members. However, project-related activities induce a bias. Therefore, a socio-economic study should reveal whether a real adoption has taken place, and under which socio-economic as well as environmental conditions this happens. In particular, the survey should help to better understand what advantages farmers recognize – these may include factors beyond easily measurable economic values, such as risk reduction, reduction of work load during planting and thinning, efficient use of local materials for seedling fertilization, etc.

Activity: The survey should consider gender, economic status, as well as meteorological scenarios (dry versus wet year). For this study in at least six villages of the intervention zone during phase 1 not only FUMA Gaskiya members should be interviewed, but also non-members, in order to detect, whether an diffusion of the technology is happening. This study should be conducted by an independent socio-economist.

Activity 2: Transfer of technology (ToT) to western Niger

Rationale: The agronomic evaluation has shown that the seedball technology works under the condition of subsistence agriculture at low mechanization level. These conditions do not only exist in the Maradi region of Niger but across the whole Sahelian belt in Niger. Already the farmer organization MOORIBEN (Fédération des Unions de Groupements Paysans du Niger, around 63 000 members, of whom 62% are women), mainly active in southwest Niger, has shown interest in this technology and sent delegates in 2018 to Maradi to gather more information.

Activity: Therefore, a technology transfer should be organised in which the farmer organisations and INRAN as local NAR-institution should play the major role. During this activity farmer exchange visits and farmer-to-farmer learning should be a major instrument, since this way a non-biased training of trainers is possible. FUMA Gaskiya has already developed radio and video spots for local use in Haussa language. These should be translated to the Djerma language that is the most prominent one spoken in SW-Niger.

Activity 3: Parallel approach to further develop and differentiate the seedball technology

Rationale: Phase 1 activities have concentrated on mechanical and chemical optimization of the seedball technology and pearl millet as a crop. However, internal testing and discussion with local stakeholders revealed additional options that could further enhance agronomic and economic viability of the technology. This concerns in particular to model the financial break even point depending on soil conditions and early season rainfall conditions, ii) the integration of fungicides into the basic recipe (such as Apron plus which has a proven positive effect on pearl millet seedling growth beyond simple protection against downy mildew), iii) the use of the technology in an integrated management concept that considers innovative low investment and labour saving innovations in the field of fertilization, weeding and pest management, and iv) the application to sorghum.
In order to avoid problems encountered during phase 1, a temporally parallel approach of agronomic and economic evaluation (by two PhD students (UH and KSU) acting in a trans-disciplinary approach is envisaged that should avoid investment in technology development that is economically not viable. These are the key activities in phase 2.

Activity 3a: Modeling the economic viability of the seedball technology based on soil and early season rainfall conditions

Already in the 2018 a master thesis on the relation between soil properties and on-farm seedball effects has started. However, results are only expected beyond the deadline of this report and sampling was temporally not optimal (i.e. after and not before the rainy season). However, for extension purposes it would be desirable to identify conditions under which the seedball technology is economically viable (i.e. forecasted yield based on soil and early rainfall conditions, and management and price level). This could be achieved by multiple linear models and a co-operation between an agronomist educated in soil science and an economist.

Activity 3b: Fungicide additives to enhance seedball efficiency

Local stakeholders proposed to include seed fungicide (Apron plus) treatment already during phase 1, but this could not be accomplished. Scientific literature (e.g. Anaso & Anaso 2010, Scheuring et al. 2008) provides evidence that an effect on early development and yield is given. Therefore, it would make sense to test this option at least in researcher managed mother plots in different villages of the Maradi area. In case of success, further distribution to farmers would need a detailed training and monitoring plan in order to avoid potential human and environmental hazards.

Activity 3c: Testing the seedball technology in a farming systems approach
The cropping of pearl millet in the Sahel is subject to multifold constraints. This begins with low fertility soils and hardly predictable meteorological conditions (e.g. start of the rainy season), concerns lacking availability of seeds, and reaches to lacking investment potential for pest management. Therefore, the seedball technology is only one piece in a farmer's pearl millet production puzzle. It can be expected that synergistic effects appear if the seedball technology was combined with other low cost but efficient management measures. Such measures are improved varieties (drought resistant, nutrient efficient), OGA (i.e. an auto-innovation by the farmer federation FUMA Gaskiya: fermented human urine to supplement N and K nutrition), micro-dosing (i.e. reduced rates of placed fertilizers per sowing pocket), as well as partial weeding (only around the sowing pockets in the first cycle to reduce wind erosion effects).

These combined options should be tested in researcher-managed mother plots in villages of the Maradi region as well as in large N-trials on-farm. While the mother plots deliver information on which combination is agronomically viable, the farmers' choice in the large-N trials allows at the same time to gather information about farmers' preferences, and to assess potential risks associated with the combined options.

Activity 3d: Developing sorghum seedballs
Pre-tests in the greenhouses of the University of Hohenheim in 2018 have shown that the seedball technology also works for sorghum if the seedball size is slightly increased. An open question is whether the chemical composition can be further improved and on-farm testing is lacking. Therefore, based on the experiences in phase 1 a shortened development frame is intended. Greenhouse trials are restricted to chemical optimization (i.e. potentially increased nutrient levels) and take into account the parallel research on fungicide addition in pearl millet and insecticide addition in sorghum (Furadan). Since on-station tests did not lead to meaningful results due to the usually over-fertilized experimental fields, direct village level testing in the Maradi region is then envisaged, using the existing infrastructure developed by INRAN and FUMA Gaskiya and the developed research procedure applied to pearl millet in phase 1 (i.e. researcher-managed mother trials, and farmer-managed baby trials).

Activity 4: Mechanization of the seedball technology
Rationale: The preceding phase has shown that the seedball technology at the given state of development mainly attracts women (about 80% of the participants). A major reason is the extensive handwork that is necessary in order to produce 10,000 seedballs per ha. The basic mechanisation options that have been developed during phase 1 have hardly improved labour efficiency. Therefore, higher level mechanization options are required that necessarily need higher economic investment. Higher investment overcharges the normal Sahelian farmer in Niger, so that group level action is needed.

Activity 4a: Mechanization development
Meanwhile a number of simple motor-driven solutions for seedball making have been developed that in simple words need only a drum, a belt, and an electric motor (e.g. https://www.youtube.com/watch?v=wDOI70IHPaM, https://www.youtube.com/watch?v=vF7PvQ0Qatg, https://www.youtube.com/watch?v=OqYTz6-zGcg). Such a solution needs to be adapted to the local conditions in villages of Niger. The major challenge is to produce seedballs of definite size and content with the given local materials, and to have a machine that can be constructed and repaired by local craftsmen, and does not present an increased risk of injury when used by laymen. In consequence, a co-operation between engineers and local actors is needed.

Activity 4b: Development of a group level action and finance plan
Definitely the investment costs for such a seedball making machine surpass the financial potentials of a typical single farm household in Sahelian Niger and call for group action. The farmer federation FUMA Gaskiya has long-term experience in supporting farmers in all kind of agriculture-related action from fertilizer purchase over seed production to participatory research. Therefore, FUMA Gaskiya is an optimal institution to develop and test a

financing model that takes into account group action, initial funding and steady costs for maintenance and repair. This activity should happen under supervision of a trained economist, who could also address the question whether seedball mechanization could be interesting for farmer-managed seed cooperatives and even for private sector seed companies.

B. Linkage to Phase I objectives and activities

The ideas detailed above directly link to phase 1 activities. In phase 1 the basic seedball technology was developed and tested in large-N on-farm trials. These activities have raised interest of the MOORIBEN farmer association of south-western Niger, which has in 2018 contacted FUMA Gaskiya to get more insight. Since soil conditions (sandy soils of low chemical fertility and water holding capacity) are very similar in parts of the MOORIBEN intervention zone, transfer of this technology appears feasible.
The ideas to test a fungicide additive with seedballs and to enlarge testing to a farming perspective were born in discussions with local stakeholders (INRAN, FUMA Gaskiya, single farmers) that see further improvement possibilities based on their own farming practices.

A particular point of discussion with men was mechanization. Men perceived seedballs as a useful technology in particular in dry years with late start of the season but the offered mechanization options were not satisfying in the sense that they are not sufficiently labour saving. Meanwhile new options appeared at the market that are from the mechanical point of view simple, i.e. manageable by farmers and repairable by local craftsmen, but they need a higher initial financial investment and a long-term strategy to finance and organize service and repair. This is a typical task for the farmer federation FUMA Gaskiya that has respective expertise, e.g. in organizing women gardening activities, where the water pump posed the same kind of problems.

Finally, farmers do not solely rely on pearl millet as a staple crop, but many practice on a minor share of their land sorghum cropping. Traditionally, sorghum is cropped on more loamy soils, but not all farmers have access to such kind of sites so that they also use sandy soils for sorghum cropping. In particular for this application, seedballs could be a supporting management option. Pre-trials in greenhouses of the University of Hohenheim have shown that seedballs work for sorghum, too, if the seedball size is slightly increased. Whether sorghum seedballs could be doped with higher nutrient content is no yet known and needs to be tested.

C. Training and outreach objectives

For phase 2, two guiding principles are important:

1. To lay more responsibility into the hands of the local stakeholders (i.e. the farmer federation FUMA Gaskiya and additionally MOORIBEN and the NAR institution INRAN).
2. To follow a real trans-disciplinary approach.

Rationale 1: Phase 1 has shown that an efficient and productive collaboration between INRAN and FUMA Gaskiya has developed, in particular mediated by the persons Maman Ali Aminou and Hannatou Moussa Oumarou. FUMA Gaskiya organised the large-N farmer participation and INRAN taught the principles of the seedball technology and supervised the application with the help of FUMA Gaskiya animators. Phase 2 activities will build on this experience.

Response 1: In phase 2 it is envisaged that animators of another farmer federation, i.e. MOORIBEN in southwest Niger are trained by joint action of FUMA Gaskiya and INRAN. Additionally, several direct farmer exchanges are planned for unbiased exchange of the addresses, not only on the seedball technology but all aspects of pearl millet and sorghum crop management. Other INRAN scientists shall be trained on the seedball technology as well, in order that this technology becomes a part of the standard portfolio.

Rationale 2: Experiences of phase 1 have shown that the effective involvement of students in the project and the respective outcome was limited. This is in particular true for the economic component. Neither at the University of Hohenheim nor at the local Maradi University sufficient interest could be raised. In addition, functional problems at the University of Maradi occurred.

Response 2: In consequence, a structural response needs to be formulated. That is real trans-disciplinarity. In phase two it is envisaged that two Phd-students - one from the field of agronomy, the other from the field of economics - work hand in hand on the scientific questions. The agronomic student is supervised by the University of Hohenheim, the economy student by Kansas State University. Both have co-supervisors at the local Maradi University.

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BIOLOGICAL CONTROL OF THE MILLET HEAD MINER IN NIGER AND SENEGAL

1 – PRINCIPAL INVESTIGATOR

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2 – RESEARCH TEAM

Co-Investigators: Ibrahim Baoua - *University of Maradi, Niger*
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Collaborators: George Norton - *Virginia Tech, USA*
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3 - PROJECT GOALS AND OBJECTIVES

Pearl millet is the world's hardiest warm season cereal crop, surviving even in the poorest soils in the driest regions and in the hottest climates. Despite this extreme climatic adaptation, pearl millet suffers from many biotic constraints including insect pests. Among these the head miner (MHM) *Heliocheilus albipunctella* (de Joannis) (Lepidoptera: Noctuidae), is a major chronic insect pest of millet in the Sahel. Over the past decades, scientists in the Sahel have identified biocontrol agents for MHM but lacked necessary resources to fully develop and scale-up biocontrol programs that could positively impact millions of millet producers. The overall objective of the project was to significantly reduce insect pest damage to millet for improvement of crop productivity. The specific objectives were to: i) Assess effectiveness of indigenous egg parasitoids for controlling MHM; ii) Optimized *Habrobracon hebetor* Say (Hymenoptera: Braconidae) rearing and release techniques for better control of MHM larval populations and; iii) Assess the economic feasibility of establishing a cottage industry for parasitoid production

4 - OVERVIEW OF ACTIVITIES

Under **Objective 1** we have carried out activities aiming at assessing the natural parasitism of eggs of the MHM and the effectiveness of identified parasitoid species. Specifically we have: i) assessed the seasonal occurrence of indigenous egg parasitoids of MHM; ii) constructed the life table and assessed demographic parameters of MHM egg parasitoids; iii) assessed host acceptance of *Corcyra cephalonica* and other Lepidoptera species for MHM egg parasitoids; and iv) assessed the intraspecific competition among the MHM egg parasitoid species.

Under **Objective 2** we have carried out activities toward improving the mass rearing technique of larval parasitoid wasp, *Habrobracon hebetor* and the on-farm releases technique of the parasitoid. We have specifically: i) evaluated the performance of *H. hebetor* when reared on *C. cephalonica* feeding on different types of medium; ii) identified the best host-parasitoid ratio for the greatest number of parasitoid offspring; iii) determined the better millet growing stage (panicle exertion, flowering, grain filling) suitable for releases of *H. hebetor*; iv) determined the better time for field release of *H. hebetor* after first appearance of MHM moths and after first record of eggs; and; v) identified optimal number of *H. hebetor* needed per acreage of pearl millet.

Under **Objective 3** we have carried out activities toward the development of a cottage parasitoid industry. We have specifically: i) conducted a baseline survey to characterize pearl millet growers in different districts in Niger; ii) assessed the economic viability of small businesses to produce and sell biological control agents and; iii) an ex-ante studies to assess the cost/benefits of mass rearing and releases of *H. hebetor.*

5) ACCOMPLISHMENTS

A. Achievements by project objectives

Objective 1 achievements: Assess effectiveness of indigenous egg parasitoids for controlling MHM

Eggs of the millet head miner have been collected in different locations of Niger and Senegal and assessed for presence of parasitism. In Senegal one parasitoid species *Trichogrammatoidea armigera* Nagaraja (Hymenoptera: Trichogrammatidae) was encountered. In Senegal, up to 37% parasitism of MHM eggs due to *T. armigera* was recorded with an average of 12%. In Niger, the parasitism of MHM eggs due to *T. armigera* ranged from 13% to 17%, with an average of 15%. Early flowering or early-planted material bore significantly more eggs of the MHM than late maturing/late flowering varieties. Newly emerging heads of pearl millet bore more eggs of the MHM than flowering heads. Contrastingly the late were significantly more parasitized than eggs of the newly emerged heads. Milky grains bore very few eggs and very low level of parasitism. In Niger, in addition to *T. armigera*, another species *Telenomus spp* was encountered on eggs of the MHM.

Host acceptance of the parasitoid *T. armigera* was tested for the rice moth, *Corcyra cephalonica*; the floor moth, *Ephestia Kuehniella*; the grain moth *Sitotroga cerealella*; the Moringa leaf defoliant, *Noorda blitealis*; the cotton bollworm, *Helicoverpa armigera*; the millet stem borer *Coniesta Ignefusalis*, and the millet head miner, *H. albipunctella*. In the laboratory, *T. armigera* successfully parasitized the eggs of the Noctuidae, *H. albipunctella* and *Helicoverpa armigera* (Hübner); the Pyralidae, *Corcyra cephalonica* (Stainton) and *Ephestia Kuehniella* (Zeller); the Gelechiidae, *Sitotroga cerealella* (Olivier); and the Crambidae, *C. ignefusalis*, and *Noorda blitealis* (Walker). However, the two crambid species had lower parasitism, and consequently, a limited number of progeny emerged from their eggs. A *T. armigera* female has a lifespan of nearly 12 days, and the development from egg to adult takes 7 days. *C. cephalonica* is the most suitable host for mass rearing *T. armigera*, having the highest parasitism levels and the highest number of progeny.

We have successfully constructed the life table of *T. armigera* and assessed the demographic parameters of the parasitoid. The male of *T. armigera* had on average 2.32 ± 0.32 days life expectancy, which could be extended to 3.38 ± 0.46 days when fed with honey. In the absence of the host species, *T. armigera* females had a lifespan of only 2.56 ± 0.33 days, which could be extended to 4.03 ± 0.11 days when supplied with honey. When continually provided with host eggs, the *T. armigera* female lifespan was extended to 11.84 ± 0.06 days. The females parasitized 13.04 ± 0.62 eggs of *C. cephalonica* per day. On average 74.06 ± 3.46% of parasitized eggs of *C. cephalonica* yielded viable *T. armigera* progeny. On average, each *T. armigera* female had a total progeny average of 106.66 ± 16.87 individuals. The development from eggs to adults took on average 7.05 ± 0.03 days. *T. armigera* progeny started emerging 7 days after parasitization of *C. cephalonica* eggs and extended up to 20 days. The sex ratio of the emerging *T. armigera* progeny was male-biased, with 2.17 times more males than females.

We have been able to conduct the intraspecific competition among the egg parasitoid *T. armigera*. Our results indicated that the addition of increasing numbers of *T. armigera* females to a given number of host eggs does not necessarily increase the parasitism. In fact in the presence of 125 eggs of *H. albipunctella*, the introduction of 1 to 30 females of *T. armigera* did not significantly affect the parasitism level. However, the number of emerging progeny did vary significantly. The number of emerging progeny/introduced parental *T. armigera* female ratio varied between 2.44-81.5 for *H. albipunctella*. We also found that the host/egg density significantly influenced the level of parasitism of *H. albipunctella* eggs by *T. armigera*. More offspring of *T. armigera* emerged when higher numbers of eggs were provided for parasitism. The offspring/host eggs ratio varied between 0.23-0.60 for *H. albipunctella* the highest being 1 *T. armigera* female for 30 eggs.

Objective 2 achievements: Optimized *H. hebetor* rearing and release techniques for better control of MHM larval populations

To optimize the current rearing technique for *C. cephalonica* (millet based diet) and to improve the production of the parasitoid *H. hebetor*, we conducted laboratory trials evaluating pearl millet, sorghum, peanut and cowpea as diet sources because these are locally grown and commonly available in Niger. Pearl millet was tested individually as well as mixed with sorghum, cowpea and peanut in the first experiment and with different portions of cowpea in the second experiment. A high number of eggs per female moth was recorded from females fed on cereals combined with legumes. Also, a high number of *H. hebetor* larvae/*C. cephalonica* larva, were produced on larvae fed on a diet of 75% pearl millet + 25% cowpea. However, more *C. cephalonica* larvae were produced in the 50% pearl millet +50% cowpea diet and as a result, this diet produced more parasitoids. Starting with 25 *C. cephalonica* larvae kept for a three-month rearing period will produce 2.7 million *C. cephalonica* larvae and 10.1 million *H. hebetor* adult parasitoids in comparison to 7.2 million *H. hebetor* on the 100% millet diet. The timely production of quantity and quality factitious host larvae is necessary for the multiplication of *H. hebetor* parasitoids in the laboratory and for field release. It is critical to have a steady supply of *C. cephalonica* larvae at the beginning of the cropping season, in early June, at the beginning of the rainy season in the Sahel, to provide cultures of the parasitoid to the farmers' cooperatives. In turn, the cooperatives need to multiply sufficient parasitoids by early August to meet the demands of the farmers for field release.

Regarding releases of the larval parasitoid, *H. hebetor*, our findings suggest that the best timing for deployment of parasitoid bags is either at pearl millet panicle emergence/flowering stage or four weeks after first sight of MHM eggs. Thus usually led to 2.5 to 7.4 times more parasitism in fields that received parasitoids compared to control fields. However, as scouting for MHM eggs could be time consuming and needs particular skills, it will obviously be much easier to use the millet phenology stage as reference for releases of parasitoids. Regarding the needed numbers of *H. hebetor* adults to be released, our results indicate that the release of either 400, 800, or 1,600 parasitoids per village led to at least twice more parasitism of MHM larvae than control villages that did not receive any parasitoids. The dose of 800 parasitoids was as effective as the 1600 parasitoids. Given the cost for producing the parasitoid one could recommend the use of 800 parasitoids.

Objective 3 achievements: Assess the economic feasibility of establishing a cottage industry for parasitoid production

Interviews with millet farmers in Niger were conducted and information gathered to help design a cottage industry and complete an ex ante impact assessment for biological control of the millet head miner in Niger. Testing of the technology followed by village focus-group discussions revealed that commercialization of the biological control technology may encounter a "free rider" problem in that *H. hebetor* in open fields will spread up to five km from its release point. This suggested the need to develop a cooperative arrangement for purchasing of parasitoids at the village level to minimize free riding.

The economic feasibility assessment provides budget analysis for the potential businesses and discusses business options for scaling, price setting, and organizing. Economic assessment indicates that a business that sells bags to 13-65 villages could expect first year profits of $50.95 - $1,996.47 if it is not subsidized by research institutions or farmer groups. Also, a business needs to sell a full set of *H. hebetor* to approximately 12 villages to cover its costs and must sell an individual bag at a price between $1.29 and $3.08 to cover the costs of outputting 13-65 bags. If individual farmers each contribute $0.09 to a cooperative purchasing agreement, a village must contain at least 557 paying households to afford a set of bags at the going rate of $3.34/bag. At $0.17 per household, a village of 295 paying households can afford a set of bags at $3.34/bag. Smaller villages can expect high, but not full, participation rates in cooperative purchases if faced with a bag price of $3.34. If three workers split income equivalent to 10% of bag revenue and a single bag costs $3.34 (which represent current business practices), a business needs to sell to a number of village buyers between 13 and 26 in order to pay workers at least $1.12/day and slightly more than 26 in order to pay workers at least $1.67/day. Farmers who use *H. hebetor* can expect to observe increased yields of 6.3 - 1394 kg depending on their farm size and the effectiveness of parasitoids, corresponding to a value of $2.08 - $460.02. A farm that produces a medium quantity of millet (900 kg) coupled with an average level of pest effectiveness (34%) and an average level of infestation (64%) could expect increased yields of 195.84 kg, corresponding to a value of $64.63.

An ex-ante evaluation of the adoption of biological control of the millet head miner by farmers in order to evaluate the net benefit has been drafted. The model included key assumptions such as: i) closed economy model (as almost 90% of the millet produced is consumed within Niger); ii) a yield improvement of 34% due to the biological control program; iii) an increase in cost per hectare estimated at 15% and; iv) a maximum adoption rate of 15% after 10 years. Preliminary economic surplus analysis resulted in a NPV of benefits over 20 years (discounted at 5%) of approximately USD 166 million.

In conclusion, the project in collaboration with a World Bank funding to University of Maradi and INRAN has facilitated in 2015 the establishment of 5 private businesses in Niger.

B. Major challenges encountered and resulting project adjustments

When the egg parasitoid, *Trichogrammatoidea armigera* was collected in the eggs of millet head miner, we could not multiply this parasitoid in the laboratory on the factitious host, rice meal moth. A technician was sent to the Biological Control Laboratory at the Cairo University in Egypt to learn the intricacies of rearing this parasitoid. Upon his return after the training, the technician was able to rear the egg parasitoid successfully on the factitious host in the laboratory. Because of the delay in mastering the eggs parasitoid rearing we have not been able to assess the interspecific competition between *T. armigera* and the 2nd egg parasitoid species *Telenomus sp.* For the same reason we have not been able to carry out the on-farm releases of the egg parasitoid *T. armigera* to evaluate its effectiveness and appropriate releases techniques.

The *H. hebetor* industry was set in motion in 2015 in Niger with a pilot program facilitated by a World Bank-funded project to University of Maradi and INRAN. The pilot program demonstrated that workers can be trained to independently rear *H. hebetor* and formulate the parasitoid bags. However, until now, as most purchases were made by NGOs, and local mayors, the sustainability of the farmers' cooperatively purchasing parasitoids has to be assessed.

The ex-ante study is yet to be completed. The Master student in charge of the study has identify the keys assumptions of the economic surplus model but has not yet completed because of lack of appropriate in-country supervision in the local university.

Two out of the three PhD students have all completed their fieldwork and submitted their first draft but got delayed because of late reviewing in the local universities.

C. Student training achievements

We have enrolled a total of 14 students of different degrees. The list, status, topics, are given in the below Table. One of the graduate student (Laouali Amadou) received BIFAD Award for Scientific Excellence in 2018.

Table 1. List of project students and topics

Student Name	Topic	University	Grade	Status
Laouali Amadou	Optimization of *Habrobracon hebetor* Say rearing and release techniques for improving control of the Millet head miner.	University of Maradi (with short training at Virginia Tech)	PhD	Will complete in 2019
Laouali Karimoune	Natural parasitism of eggs of the millet head miner and efficiency of *Trichogrammatoidae armigera* in biological control	University of Maradi (field work at ICRISAT)	PhD	Will complete in 2019
Mame Fatoumata Goudiaby	Diversity of pearl millet insect pests and their biological regulation in the Senegal groundnut belt	University Cheikh Anta Diop de Dakar	PhD	Will complete in 2019
Michael J. Guerci	Economic Feasibility of a Biological Control Cottage Industry in Niger	Virginia Polytechnic Institute and State University	MSc	Completed in 2016
Oumou Moumouni	Ex ante impact assessment of the biological control of millet head miner in Niger	UAM-Niamey (with short training at Virginia Tech)	MSc	
Hamidou Leyo Idrissa	Biological control of the millet head miner by direct releases of different numbers of the parasitoid Habrobracon hebetor in 12 villages of the Tillaberi region in Niger	UAM-Niamey (field work at ICRISAT)	MSc	Completed in 2017
Saidou Amani Laminou	Identification of optimal timing for releases of Habrobracon hebetor for biological control of the millet head miner in the Tillaberi Region	UAM-Niamey (field work at ICRISAT)	MSc	Completed in 2017

Issoufou Chaya Mahaman Kabirou	Evaluation of natural parasitism of the millet head miner in the Tahoua region.	University of Maradi (field work at INRAN)	Bsc	Completed in 2017
Arzika Lele Mahaman Zaharadine	Bio ecology of *Corcyra cephalonica*, host species for mass rearing *Habrobracon hebetor* Say.	University of Maradi (field work at ICRISAT)	BSc	2017
Amadou Yari Harouna	Identification of optimal timing for releases of Habrobracon hebetor for biological control of the millet head miner in the Tahoua Region	University of Maradi (field work at INRAN)	BSc	2017
Bay Ndiaga Thiam	Effectiveness of augmentative releases of Habrobracon hebetor against the millet head miner in the Senegal	University of Thies, Senegal (field work at ISRA)	Bsc	2017
Abba Ousmane Abdoul Razak	Effect of different density of *Habrobracon hebetor* Say for controlling the millet head miner.	University of Maradi (field work at INRAN)	Bsc	Completed in 2016
Hamidan Barka Chiitou	Effect of different density of *Habrobracon hebetor* Say for controlling the millet head miner.	University of Maradi (field work at INRAN)	Bsc	Completed in 2018
Abdou Mato Rahamatou	Evaluation of *Trichogramma armigera* effectiveness against the millet head miner.	University of Maradi (field work at INRAN)	Bsc	Completed in 2018

D. Short-term training and outreach

We have trained technicians from ICIPE, Kenya and from INERA, Burkina Faso on mass multiplication of *Corcyra cephalonica* and *Trichogrammatoidea armigera*. We have also provided training to farmers/extension agents on the biological control program of the millet head miner. The list is provided in below table:

Table 2. List of trainings provided to farmers and extension agents

Year	Topic and type of attendees	Country	Male	Female
2014	In situ training of farmers on millet head miner, and the biological control program	Niger	160	80
2015	In situ training of farmers on millet head miner, and the biological control program	Niger	317	49
		Senegal	204	100
	Training of farmers cooperative on rearing of factitious host, parasitoid wasps and formulation of releases bags	Niger	17	1
2016	In situ training of farmers on millet head miner, and the biological control program	Niger	731	131
		Senegal	115	6
	Training of farmers cooperative on rearing of factitious host, parasitoid wasps and formulation of releases bags	Niger	27	2
2017	In situ training of farmers on millet head miner, and the biological control program	Niger	412	38
	Training of farmers cooperative on rearing of factitious host, parasitoid wasps and formulation of releases bags	Niger	19	2
2018	Training of farmers cooperative on rearing of factitious host, parasitoid wasps and formulation of releases bags	Niger	16	2
Total			**2018**	**411**

6 - UTILIZATION OF RESEARCH OUTPUTS

A. Technologies and management practices by phase of development

Managements and cultural practices Phase 3:

1) We have fine-tuned the release technique of *H. hebetor*. We recommend release of 800 parasitoids per 3km radius in the early panicle stage of the crop to obtain maximum percentage of parasitism and control of MHM. If parasitoids are to be released using the jute bag technique, the 800 parasitoids correspond to 12 parasitoid bags. This will reduce the current numbers by (20%) for 3km radius. Given the current price of $3.34/bag, a saving of $10 is expected per each release. The technology is available for transfer to farmers' cooperatives in Niger and will be discussed with donors and USAID value chain projects to support the dissemination. 2) We have also

fine-tuned the rearing technique of the factitious host *Corcyra cephalonica* and the parasitoid *H. hebetor*. We are recommending replacing the 100% millet diet by a mixture of 50% pearl millet +50% cowpea diet for mass culturing *Corcyra cephalonica* the host on which the parasitoids are being multiplied. This will lead to 43% increase of the parasitoid population within a three-month rearing period. The technology is available for transfer to farmers' cooperatives in Niger and will be discussed with donors and USAID value chain projects to support the dissemination. 3) We have mastered the mass culture of *T. armigera* and developed a standardized rearing method. We are recommending giving 30 eggs of *C. cephalonica* for parasitism by one *T. armigera* female for 6 days followed by another batch of 30 eggs for the remaining 6 days of their life. The females will have to be given new males to mate with every 3-4 days for a higher ratio of females in the progeny.

Management and cultural practices Phase 1:

The head miner egg parasitoid *Trichogrammatoidae armigera* is being tested for use as a biological control agent in addition to the larval parasitoid. At current stage of research we have successfully established colonies and developed a standard mass culture technique for the parasitoids on factious host *Coryra cephalonica* and effectiveness against the MHM in the laboratory has been established. This technology was developed in the later stage of Phase I and needed on station/on-farm testing to validate the potential of the egg parasitoid against the millet head miner.

B. Intermediate outputs

The *H. hebetor* industry was set in motion in 2015 with 5 private units. Development projects, NGOs in Niger were involved in purchase of parasitoid bags and distributing them to the farmers. The 5 private units have been operating for 3 years and needed to be assessed in order to evaluate and to assess how many farmers have been affected by the parasite release, how much of an effect it has had, and the challenges to advance the business.

A. Publications

The project achievements have been published in the following papers:

Karimoune L., Ba N.M., Baoua I.B., Muniappan R. (2018). The parasitoid Trichogrammatoidea armigera Nagaraja (Hymenoptera: Trichogrammatidae) is a potential candidate for biological control of the millet head miner Heliocheilus albipunctella (de Joannis) (Lepidoptera: Noctuidae) in the Sahel. Biological Control 127, 9-16.

Guerci MJ, Norton G, Ba NM, Baoua I, Alwang J, Amadou L., Moumouni O, Laouali K, Muniappan R (2018) Economic feasibility of an augmentative biological control industry in Niger. Crop Protection 110, 34-40.

Amadou L., Baoua I., Ba M.N. and Muniappan R. (2018). Development of an optimum diet for mass rearing of the rice meal moth *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) and production of the parasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae) for the control of pearl millet head miner. Submitted

Amadou L., Ba M.N., Baoua I., Muniappan R. and Sidhu J. (2018). Timing of releases of the parasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae) and numbers needed in augmentative biological control against the millet head miner *Heliocheilus albipunctella* (de Joannis) (Lepidoptera: Noctuidae). Submitted

Goudiaby M.F., Sarr I., Sembene M and Ba M. Efficacy of augmentative release of *Bracon hebetor* against the pearl millet headminer in Senegal (In preparation)

7 - STATEMENT ON PROPOSED FUTURE ACTIVITIES FOLLOWING PHASE I RESEARCH ACCOMPLISHMENTS

A. Statement on future activities in Phase II

1. In Phase II, we will streamline mass production of the egg parasitoid *Trichogrammatoidea armigera* (Hymenoptera: Trichogrammatidae) in the laboratory on the eggs of the factitious host rice moth, *Corcyra cephalonica* (Lepidoptera: Pyralidae). Unlike the larval parasitoid *Habrobracon hebetor* (Hymenoptera: Braconidae), this parasitoid is a weak flier and spreads over only a short distance. We need to determine the number of parasitoids to be placed per hectare for optimal parasitization. We also need to identify the appropriate method for releases of the parasitoids (cards glued with parasitized eggs, direct releases of adults). We will also explore the possibility of cold storage of parasitized/unparasitized eggs for accumulation of numbers needed for releases. Ants and other predators are known to attack eggs in the field. Appropriate technologies need to be developed to prevent predation on the parasitized eggs placed in cards in the field depending upon the predators observed.

2. In Phase I, mass production in the laboratory, number of parasitoids to be released per a given area, time of release and privatization for field release for the larval parasitoid, *H. hebetor* have been worked out. In the phase II, field release of combined egg and larval parasitoids will be tested with the following treatments: 1) Control, 2) Release of egg parasitoids alone, 3) Release of larval Parasitoids alone, and 4) Release of both egg and larval parasitoids.

3. We also need to explore the potential of the 2nd egg parasitoid, *Telenomus sp* against the head miner both in the laboratory and the field and the interspecific competition with *T. armigera.*

4. We have established private parasitoid units and need to assess their effectiveness and make necessary adjustments including the business opportunities for sustainability. This analysis will include the integration of other products (i.e. neem) to widen the business opportunity of the farmers' cooperatives.

5. With the on-going threat caused by the fall armyworm, *Spodoptera frugiperda* to many crops including sorghum, the current approach which only focuses on maize could even worsen the situation on sorghum which is so far the 2nd preferred host plant in Africa. In addition to that the current orientation relies mainly on pesticides for which the fall armyworm could evolve resistance. We are suggesting an integrated approach, which address the pest both on maize and sorghum. The parasitoid rearing/release platform we developed for millet head miner during Phase I could be used for identification/assessment of locally recruited parasitoid of the fall armyworm for its control on sorghum.

B. Linkage to Phase I objectives and activities

The proposed activities on testing of *T. armigera* effectiveness on station and on farm are a continuation of Phase I Objective 1, which got delayed because of late establishment of eggs parasitoids culture. Likewise combination of egg and larval parasitoids need to be evaluated for an integrated control approach in liaison with both Phase I Objective 1 and 2. The activities on the 2nd egg parasitoid, *Telenomus sp* are a continuation of Objective 1 of phase 1.

Objective 3 of the Phase I, assessment of economic feasibility of establishing a cottage industry of larval parasitoid production has been completed. In the Phase II, impact assessment of release of egg and larval parasitoid release for control of MHM and benefit to the farmers will be assessed. Similarly, the functionality of the businesses will be assessed.

C. Training and outreach objectives

1. Transfer of eggs parasitoid technology to farmers' cooperatives

2. PhD and MS students will be supported for studies in Universities in Niger/Senegal with short training at Virginia Tech.

3. Undergraduate students from the University of Maradi will be involved in laboratory and field studies.

4. Members of Farmers Cooperatives will be trained in eggs parasitoid production and field release.

EXPANDING MARKETS FOR SORGHUM AND MILLET FARMERS IN WEST AFRICA THROUGH STRENGTHENING OF ENTREPRENEUR PROCESSORS AND NUTRITION-BASED PROMOTION OF PRODUCTS

1 – PRINCIPAL INVESTIGATOR

Bruce Hamaker – *Purdue University, USA*

2 – RESEARCH TEAM

Co-Investigators: Mario Ferruzzi – *North Carolina State University, USA*
Moustapha Moussa – *INRAN, Niger*
Cheikh N'Diaye – *ITA, Senegal*
Djibril Traore – *ITA, Senegal*

Partners: McKnight Foundation
Food Processing and Post-harvest Handling Innovation Lab (FPL)
Education and Research in Agriculture (USAID, ERA)

3- PROJECT GOALS AND OBJECTIVES

The activities of this project expand work with entrepreneur processors through local incubation centers (now termed "Hub-and-Spoke Food Processing Innovation System") with strategies for developing new extruded and other products, innovative ways to promote sorghum and millet processed products, and nutrient fortification of products that is sustained through market demand. The specific project objectives include:

1. Introduction of new grain processing technologies and development of new sorghum and millet-based products.

2. Enhance nutritional quality aspects of sorghum and millet foods to drive markets and improve food security and nutrition.

3. Incubate sorghum and millet entrepreneur processors in Niger and Senegal.

4. In Niger, jointly with the McKnight Foundation project, promote sorghum, millet and legume processing at the rural level to gain market access for smallholder farmer women and improve food and nutrition.

5. Conduct long-term and short-term training programs to support institutional capacity building.

6. Take proactive steps to take into account gender-specific constraints, preferences and practices in project activities in order to assure equitable participation and input from male and female farmers, processors and consumers.

The research team aims to create successful models using food and nutrition-related technologies to expand markets and improve nutrition and health of vulnerable groups, and to have these models be used elsewhere. Scientific and technological research is being used to generate advancements in sorghum and millet utilization while capacity building is incorporated through short-term and graduate degree training.

4 - OVERVIEW OF ACTIVITIES

In our Phase I SMIL project, most notably we have created a new model of disseminating food processing and nutrition technologies to rural Africa that generates income, empowers women, and addresses rural nutrition issues. This activity has matured over a number of years in Niger, starting as an urban entrepreneur incubator in Niamey with the USAID INTSORMIL CRSP program, but with SMIL, and in partnership with the McKnight Foundation, has developed into a more expansive rural development model, which we call the "Hub-and-Spoke Food Processing Innovation System" (Figure 1). The food processing technology Hub is comprised of millet, sorghum, and legume processing equipment to produce high quality consumer products for the marketplace and includes support from a professional staff made up of food technologists, a nutritionist, an economist, communication specialists, and a mechanic to provide technical training, backstopping, and R&D function to rural and urban Spokes. These Spokes consist of entrepreneur processors and rural women's associations who start and sustain successful enterprises. It is a post-harvest development tool designed to empower women and youth, generate incomes, and improve nutrition in rural and urban communities. A larger goal is to provide better markets for local farmers and to use this system to disseminate insights on food-based nutrition strategies. In Niger, SMIL added extrusion technology, through the addition of a low-cost, robust small-scale (25-35 kg throughput/hour) extruder, and work has been done to process instant flours for thin and thick porridges, couscous, and the local beverage *fura*. These have been studied to improve product quality (i.e. millet varietal differences, extrusion conditions, use of whole grains, and extrusion of composite blends of cereals with nutrient-rich plants), sensorial acceptability, and consumer preference. Finally, a large market study is currently being completed on instant millet flour for thick porridges and for the *fura* product. In collaboration with T. Dalton and his student T. Nakelse, studies were done on nutritional labeling and consumer attitude, products with best market potential, the potential market size of processed millet/sorghum-based products, and willingness-to-pay for products. In the rural Spoke innovation centers (put in place in 4 locations in Niger and 1 in Burkina Faso), women associations have been given detailed training in processing of millet-based foods, have established viable rural markets and have documented profits, and have in each location taken it under their own initiative to train women from nearby villages peripheral to their centers.

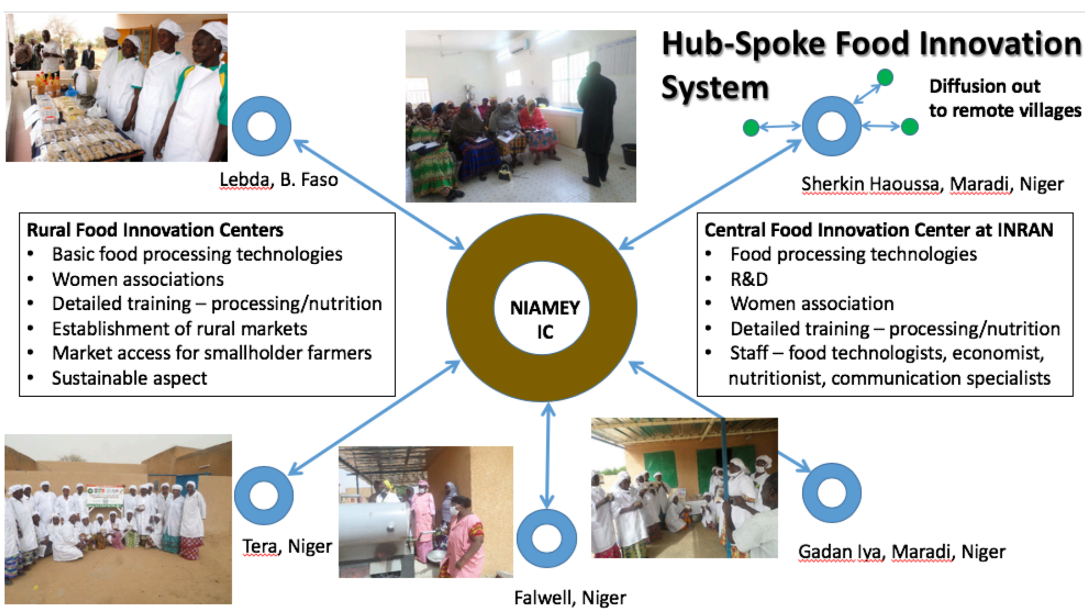


Figure 1. The Niger Hub-and-Spoke Food Processing Innovation System.

Nutrition has become a focus of our work, and natural fortificants [shortfall micronutrients (iron, zinc, pro-vitamin A) and protein] have been used to make low-cost foods in these rural communities with evidence of repeat buying, and a consumer study with a total of 320 mothers showed certain local fortified millet blends are preferred over food-aid blends.

In Senegal, new millet products were formulated, including extruded whole grain millet instant flours for direct sale for thin and thick porridges and as an ingredient in commercial bakeries, instant *arraw*, and fermented "economic couscous", a low-cost alternative to conventional couscous processing and using a Lactobacillus planetarium strain developed by Belgian collaborators of ITA. Sensory studies showed high acceptability for the products. Dakar entrepreneur processors were trained and products were tested. In collaboration with FPL and ERA, many other processors were trained in Phase I from various regions of Senegal.

5 - ACCOMPLISHMENTS

A. Achievements by project objectives stated in your proposal

1. **Introduction of new grain processing technologies and development of new sorghum and millet-based products.**

Achievements:

Extrusion technology was introduced at INRAN/Niger with the purchase, installation, and training of a small-scale (25-35 kg throughput/hour) extruder developed by engineers at Purdue University. In Senegal at ITA, the same extruder had been installed in the last part of the INTSORMIL CRSP project. In both locations, new (mostly) millet products were developed during Phase 1 that included 1) in Niger, high quality decorticated and whole grain instant flours for thin and thick porridges, extruded couscous, and the local millet beverage *fura*, and 2) in Senegal, further development and refinement of "economic couscous" (a product that avoids the agglomeration process to make couscous particles and is lower in cost) to make consistent quality fermented economic couscous, a fortified infant food, instant *arraw* using a carbohydrate-based binding agent, and instant whole grain flours, all for the Dakar market.

In Niger, all extruded products were newly developed in the SMIL Phase I project. This included instant millet flours for thin and thick porridges that, while existing in commercial markets elsewhere in more industrialized African countries (e.g. South Africa), were not locally made in Niger and using a low-cost extruder. A new millet couscous product technology was developed by the INRAN team that allows approximately 10x the amount made per day compared to that which 5 women can make (~300 kg/day versus ~30 kg/day), and consumer sensory tests on different millet varieties showed that some varieties make extruded couscous with quality equal to high quality hand-agglomerated millet couscous (Figure 2). Finally, a new instant millet *fura* product was developed and consumer preference and willingness-to-pay (WTP) studies were completed showing good potential for *fura* as a commercial product. *Fura* is a popular thin porridge beverage product in the region (Niger, Nigeria, Burkina Faso). It is prepared with decorticated millet flour, whereby large particles (boulettes) are made and steamed, then pounded and the boulettes are again made to the final product. This is a lengthy process often taking 2 days to complete.

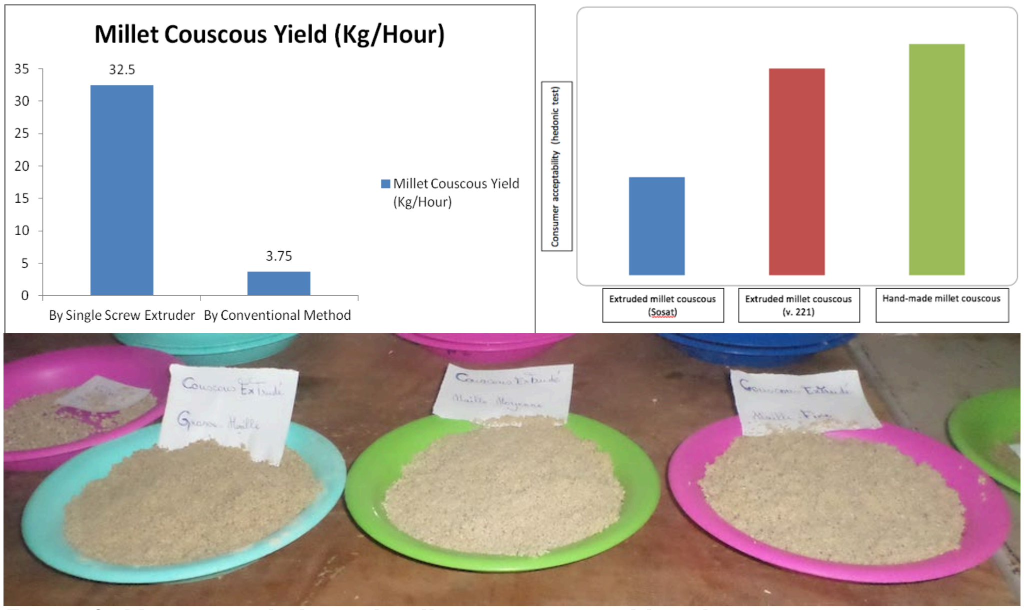


Figure 2. New extruded pearl millet couscous yield and consumer acceptability compared to traditional hand-agglomerated couscous. *Daily amount processed was 10x higher for the extruded product and, depending on variety, acceptability was equal to high-quality traditionally-made couscous (red bar). Photo shows extruded couscous made from 3 millet varieties.*

The processing technology was developed with the extruder to make instant fura at the high throughput capacity of the extruder (~200 kg/day). Consumer preference data showed both decorticated and whole grain millet instant fura products were accepted as well as the traditionally prepared fura products, though this was dependent on the millet variety used (8 varieties were tested, in partnership with the McKnight Foundation project) and on the location tested (3 locations with 2-3 villages in each). Willingness-to-pay data showed a willingness to pay for the extruded fura of 155 CFA (~$0.25)/500 g more than traditional fura. This was attributed the faster preparation of the product by the consumer. Currently, a large market study is being completed on instant millet flours for thick porridges and instant fura in Niamey. Urban processors in the association linked with INRAN were trained in distribution and promotion of extruded products as part of the preparations for the market study.

At Purdue, laboratory work was completed (M. Moussa, PhD student) on consumer-related properties of extruded couscous prepared at INRAN using 8 millet varieties. Size-exclusion chromatography analysis of the starch component showed the presence of a low molecular size fragment in the extruded couscous, which seemed to translate to a smoother texture of this couscous compared to the traditionally prepared couscous. Laboratory data supported the sensory testing that showed extruded millet couscous is accepted equally well as traditionally prepared couscous. Millet variety was a factor for acceptance and varied in laboratory analyses. Thus, the extrusion technology (single-screw small-scale extruder) is an innovation in making high quality, high yielding couscous with commercial potential.

In Senegal, extruded instant millet flour was successfully used in combination with binding agents (maltodextrin, xanthan gum) to process instant arraw, a popular agglomerated product. This is the first time a commercially-viable instant arraw product has been made. Tests showed a substantial decrease in arraw production time. Also, cooking times were reduced for the two arraw diameters: from 24 to 10 min for the 2 mm diameter and from 35 to 15 min for the 4 mm diameter products. Prototype products were shown to a major entrepreneur processor in Dakar and high interest was expressed. Also, at ITA, fortified instant whole grain millet products were formulated and sensory studies were begun on understanding how whole grain products can be made acceptable to the Senegalese urban consumer, who are not accustomed to whole grain foods. Work on "economic couscous" a less energy intensive and lower cost couscous process developed in the last years of the INTSORMIL project, was advanced with studies on fermented economic couscous using Lactobacillus strains developed by ITA with the Wallonie region cooperation (Gembloux, Belgium). Dakar millet processors working with ITA and the SMIL project see a good market for such a fermented couscous product and the necessary consistency and quality can be obtained using a pure culture fermentation. Also, there appears to be a good market for such a product in the Senegalese diaspora in Europe and North America. Six samples of fermented economic couscous were tested for sensorial acceptability using 85 panelists. Fermentation level, taste, and overall acceptability were measured. From this two fermented couscous samples were determined to have the highest acceptability and preferred taste/fermentation level.

2. **Enhance nutritional quality aspects of sorghum and millet foods to drive markets and improve food security and nutrition**.

Achievements:

Screening of local micronutrient-rich plant materials was completed with approximately 20 samples in both Niger and Senegal. In complementary work to this project, the FPL micronutrient team at Purdue/North Carolina State University and University of Pretoria showed that baobob extract added at as little as 5% w/w increased bioaccessibility of iron, zinc, and ultimately not negatively impacting pro-vitamin A carotenoids. FPL will test this finding in a human study in the next project year.

Figure 3. Extruded composite blends of whole grain millet, baobab and moringa.

Selections were made based on nutrient content for formulation into fortified millet products which were tested for extrusion at Purdue (Figure 3). These include iron sources (moringa leaf and baobab), as well as protein (peanut and tigernut).

Training was provided by INRAN Incubation Center, by the nutritionist and food scientists, in the summer of 2017. Also, PI's Ferruzzi and Hamaker gave training in nutritional aspects of natural fortificants and millet-based foods.

In Niger, in partnership with the McKnight Foundation project, naturally fortified formulations were made using improved millet and legume varieties and micronutrient-rich plants (moringa, baobab fruit, carrot, pumpkin). Four formulations were developed and tested in 3 rural locations (total of 8 villages) and compared with food-aid conventionally fortified blends (Misola - millet/cowpea/premix, WFP - maize/soy/premix). Panelists consisted of 320 mothers in the 8 locations and preference testing was done. Three improved millet varieties were used [ICRISAT and INRAN provided the varieties that were grown by the farmer organizations (Fuma Gaskiya and Mooribeen) at the different locations - 89305 for Falwell, PPBTERA for Tera, 99001 for Maradi), and compared to a local millet variety. The cowpea variety was TN378 and peanut was 55437 (previous studies were shown that these varieties were best accepted by the local mothers and children). The base formulation used 60% millet, 25% cowpea, 15% peanut, and the other 3 formulations reduced millet to 50% and varied the other 10% in composition depending on location in amount and proportion of moringa, baobab fruit, carrot, and pumpkin. Results showed that overall acceptability of the fortified products was good and better in some cases than the food-aid products (Figure 4). Also, the formulated naturally-fortified millet products of the project generally had markedly lower variability among subject's response.

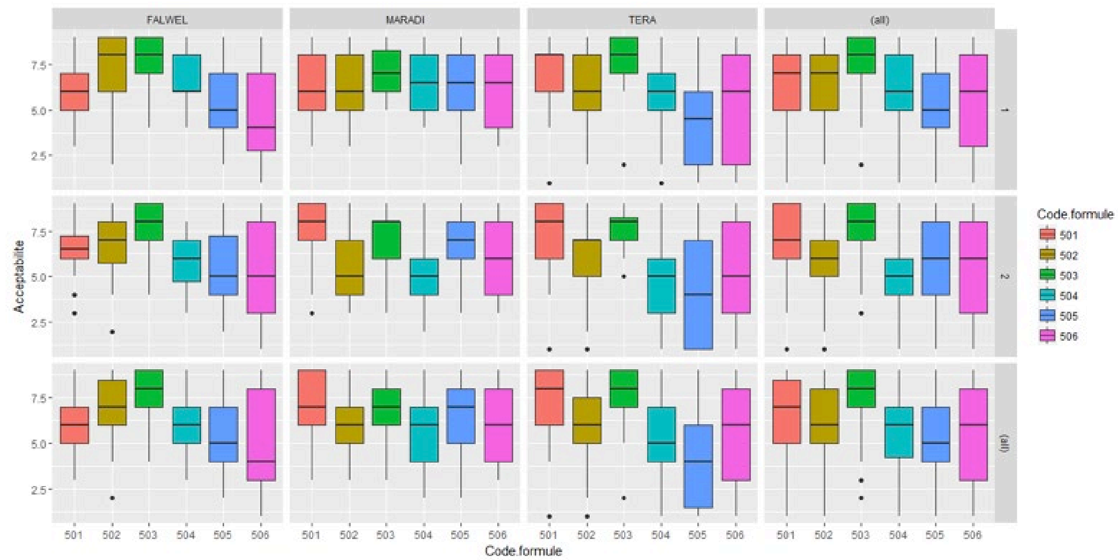


Figure 4. Consumer preference test of naturally fortified flours formulated locally with whole grain millet-cowpea-peanut-minerals/vitamins compared to cereal-legume-based food-aid blends (Misola and WFP) in 8 villages (associated with Falwel, Maradi, Tera) in rural Niger. *Formula legend: local (501-504); 501=whole Ggrain (millet-89305)-cowpea (TN378)-peanut (5544); 502= whole grain (millet-99001)-cowpea (TN378)-peanut (5544)-moringa-baobab; 503=whole grain millet (PPBTera)-Ccwpea (TN378)-peanut (5544)-Carrot-baobab; 504=whole grain millet (local variety)-cowpea (TN378)-peanut (5544)-moringa-pumpkin; and food-aid blends (505-506); 505=Misola (millet-soybean-peanut-vitamin/mineral premix); 506=WFP (maize-soybean-vitamin/mineral premix) (n=320, $P<0.05$).*

Studies were done, with partial support from SMIL, to understand factors in millet (and sorghum) that cause a satiety response (sustained energy and fullness). A study was done with collaborators at University of California, Davis, using a gastric simulator, that showed millet couscous breaks down into small cooked particles in the stomach that appear to form a viscous "plug" which may slow stomach emptying. We previously had shown, during the INTSORMIL project, that millet and sorghum foods (thick porridges and couscous) have significantly slower stomach emptying than other starch staple foods. This is the reason that millet and sorghum foods are satiating and give people sustained energy and fullness after consumption. We are interested in using this attribute to market these foods.

3. Incubate sorghum and millet entrepreneur processors in Niger and Senegal.

The main output of the project in the area of incubation of entrepreneur processors and women associations was the development and maturing of the Hub-and-Spoke Food Processing Innovation System, as introduced above in 4) Overview of Activities. In Niger, in partnership with the McKnight Foundation, an already well functioning incubator for urban processors in Niamey was developed to organize rural women associations and to initiate processing activities to serve and grow rural markets, generate incomes, and improve nutrition through developing nutrient-fortified foods that kids and other ages really want to eat and can buy at a low-cost point. Good documentation of the activities of the 5 rural Spoke Centers show modest, but significant profits that are having a transforming effect in households and at the local village/town community level. In the Falwel, Niger site, total sales and net profits are shown in Figure 5. Table 1 shows the range of products and amount processed and sold at the Falwel innovation center.

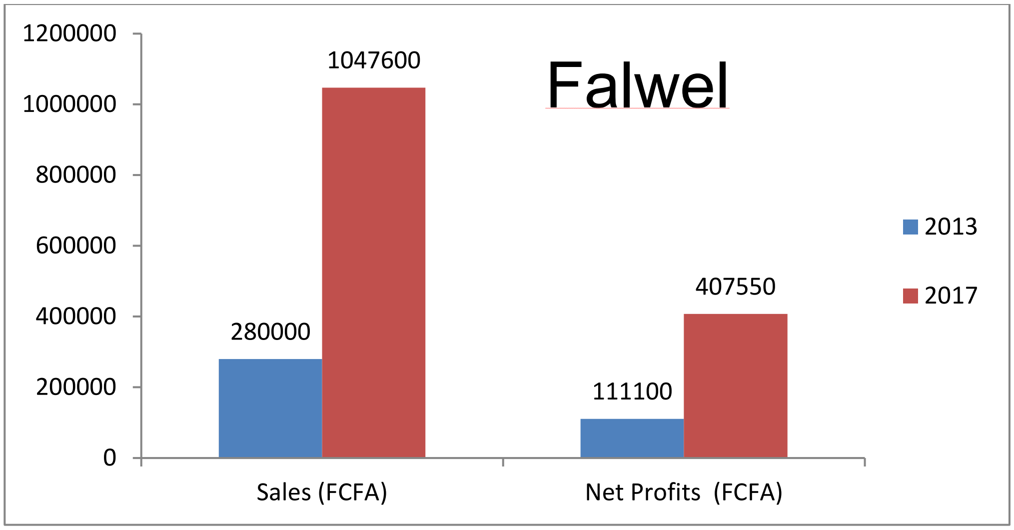


Figure 5. Total processed product sales and net profits at the Falwel site in 2013 and 2017.

Table 1. Type of processed products sold by the Falwel women's association in the 2013-2017 period.

Processed Products	**Annual Sales (FCFA)**		
	2013	**2015**	**2017**
Millet Dégué	96000	384000	288000
Sorghum Dégué	24000	96000	0
Millet Couscous	128000	72000	64000
Sorghum Couscous	32000	64000	0
Cowpea Couscous (*béroua*)	0	270000	405000
Cowpea Flour	0	0	22500
Fortified Millet Flour	0	0	35000
Peanut Oil	0	0	63000
Peanut Cake (*tourteau*)	0	0	233100
Total	**280000**	**886000**	**1110600**

We have identified the core aspects or pillars of the Hub-and-Spoke system that we believe can be used in other locations to scale this technology incubator, and are listed as follows.

Pillars are 1) the central "Hub" food innovation center, 2) rural "Spoke" food processing and innovation centers, 3) communication between Hub and Spoke teams, and 4) data collection and documentation.

Hub Food Innovation Center

Essential requirements:

- Support by the host institution
- Personnel – food scientists/technologists, equipment engineer/mechanic
- Value-chain and identification of products with market potential
- Processing equipment – the entire line or lines to make high-quality commercial products

- Urban processors group linked with Hub
  - Participate in product testing and promotion
  - Provide guidance and backstopping to rural processors
- Training staff with expertise in:
  - Processing
    - Food safety and sanitation
    - Packaging and labeling
    - Equipment maintenance
    - Business skills (management, marketing, distribution, bookkeeping)
- Research and Development (R&D) function
- Business plan development

Rural Spoke Food Processing and Innovation Centers

Essential requirements:

- Creation of women/youth associations/groups
  - Linkage to local farmer organizations, and with access to improved varieties
- Support by community authorities (e.g. chief, mayor) and families
- Hub to give full responsibility and leadership in operation to rural women's association (groups)
  - Establishment of contract of partnership (MOU) between Hub institution and women's association for operational funding
- Access to commodity production site
- Business plan development

Communication between Hub and Spoke Teams

Essential requirements:

- Transportation to exchange information and technologies
- Information and communication technologies (ICT) (e.g. WhatsApp, email, etc.)
  - Urban-urban
  - Urban-rural
  - Rural-rural
- Periodic follow-up and monitoring of on-going activities

Data collection and documentation

Urban entrepreneur processors in both Niger and Senegal were involved in the incubation activities involving the extruder. In Niger, training sessions were given on product development, extrusion and other processing technologies, finance and business management, product distribution, and leadership. Training was given on incorporation of nutrient-rich ingredients into processed foods, including baobab, moringa and others. Urban processors were also involved in the use of improved labels on millet and sorghum products. Also provided them with training on food safety and processing. Mme. Liman, the lead processor in Niamey, received approximately 180,000 USD from UKAID that was facilitated by INRAN/SMIL, and purchased the following equipment - mills, decorticators, electric dryer (capacity of 1 MT/day), packaging equipment (capacity of 1 MT/day), couscous steamers. Her processing facility was also expanded to include a third floor. A board of directors was established for the Enterprise de Transformation Cerealiere (ETC). She also increased the staff and increased her point-of-sales stores to 50. In Senegal, Dakar processors at Free Work Service and Maria Distribution worked closely with ITA to develop products that they see have new commercial opportunities (extruded whole grain instant flours for thin and thick porridges, instant arraw, and a new fermented "economic couscous" described above).

In February 2018, two officials from the African Development Bank visited Niger with M. Moussa and B. Hamaker to observe the incubator Hub-and-Spoke Food Processing Innovation System and the extruder technology. This was following attendance by both M. Moussa and B. Hamaker at the previous held High Ministerial Dialogue to support the Post-Harvest Losses Reduction and Agro-Processing (PHAP) Flagship Launch at the African Development Bank headquarters in Abidjan on November 21-22, 2017. B. Hamaker gave the opening keynote address on the importance of post-harvest activities in development and use of the food processing Hub-and-Spoke model to promote rural and urban agricultural development, income generation, women and youth empowerment, and improved nutrition. During the visit, a training session was held on the extruder technology, nutrition, and distribution and promotion of extruded products prior to the market study.

4. **In Niger, jointly with the McKnight Foundation project, promote sorghum, millet and legume processing at the rural level to gain market access for smallholder farmer women and improve food and nutrition.**

The interaction and synergy between SMIL and the McKnight Foundation project has continued to grow and expand. An important network has been established that supports farmers and processors (rural and urban), research scientists, students, extension and development personnel, policy makers (mayors, central government), private sector food processing industries, seed producers, communication specialists, nutritionist, equipment fabricator, local business consultants, and funding institutions.

Although we do not yet have donor funding to replicate the hub-and-spoke incubation model, we do see that the processing/nutrition knowledge gained by the rural women's associations is naturally spreading to secondary regional villages (distances vary from 10 to 30 km from the rural incubation site). In almost every rural Incubation Center site, the women's associations have taken it upon themselves to invite, and sometimes pay, for women to travel from regional villages to be trained in food processing and nutrition technologies. This dissemination of knowledge now includes from 7 to 11 villages at each rural incubation site (Falwel, Tera, Sherkin Haoussa, Gadan Iya in Niger). As an example, the Falwel site trained 242 other women and youth in 2017, from an association of 42 women.

5. **Conduct long-term and short-term training programs to support institutional capacity building.**

Moustapha Moussa (Niger) is in his final year of his PhD studies with B. Hamaker at Purdue University. He completed his PhD preliminary examination and is done with his coursework. He has 3 draft manuscripts - one on millet couscous, a second on the Hub-and-Spoke system [presented at the International Sorghum Conference (ISC) in Cape Town], and a third on natural fortification of local millet-based blends. He is a PhD candidate at Purdue University and has the expectation to finish his degree program in the summer of 2019. Anna Hayes, PhD student at Purdue, was partially funded by SMIL when completing her studies on the fullness and satiety attribute of millet-based products, in particular millet couscous. She is preparing a manuscript on her study, and was part of a paper published by our group in the past year (Cisse et al., Nutrients 2018; see Supporting Documents).

Cheikh N'Diaye (Senegal) completed his PhD at Purdue under the direction of Prof. Mario Ferruzzi and Bruce Hamaker (co-advisor) through the support of the Food Processing Innovation Lab (FPL) and the USAID Education and Research in Agriculture (ERA). He is now Principal Investigator at ITA in Senegal for the SMIL project. Hawi Debelo (Ethiopia) was partially supported by SMIL in her PhD studies and will defend her thesis in November 2018.

6. Take proactive steps to take into account gender-specific constraints, preferences and practices in project activities in order to assure equitable participation and input from male and female farmers, processors and consumers.

Work was with principally rural and urban women processors and now with more emphasis on youth. In Niger, women associations of the rural food innovation centers have, in every case, taken it under their own initiative to train other women in food processing to gain incomes from rural markets, as described above. Consumer studies are either directed at both women and men, or when the target group is children then mothers are recruited.

B. Major challenges encountered and resulting project adjustments

In Niger, our SMIL project has been fortunate to have had the partnership with the McKnight Foundation to develop and mature the Hub-and-Spoke model for incubating rural women processor associations, and to reach a stage where we believe the system is scalable. It is a synergy in the true sense where more has been achieved by both parties than could have been made were the project working apart. There have been challenges, though none have been large and had to result in significant project adjustments.

In Senegal, we accomplished less, though made significant progress in working on new products with the extrusion technology and "economic couscous" technology developed in the last part of the INTSORMIL program. A challenge with the PI (D. Traore) at ITA was to meet objectives and targets in a quantitative manner. Still, progress was made in the promotion of both the FPL and SMIL projects at the Senegal government level, and with their partners at the World Food Programme and NGOs, and the ITA PI was largely responsible for that. In the final year of SMIL, we replaced PI Traore with a new PhD graduate from Purdue, Dr. Cheikh N'Diaye, and quantitative experiments are showing very good results as well as interactions with urban entrepreneur processors. A second phase of the project in SMIL, as discussed below, would additionally have a closer synergy with the FPL project and successes that are being had in rural processing of fortified instant millet flours and scaling-up of these activities to other regions of Senegal.

C. Student training achievements

See Accomplishments #5 above, which describes student training in this SMIL project. Many local university students have been trained both in Niger and Senegal, and this data can be found in the SMIL reporting system.

D. Short-term training and outreach

One of the important aspects of working with entrepreneur processors in this project is training, and many training sessions have been done over the 5 years, including training in processing technologies, formulations, nutritional fortification (natural plant materials), business skill development, marketing, and other.

6 - UTILIZATION OF RESEARCH OUTPUTS

A. Technologies and management practices by phase of development

The introduction of extrusion technology in Niger and its further work on it in Senegal has been successful in generating high quality consumer-desirable products, and created a lot of interest from entrepreneur processors, the governments, and NGOs. We see that the array of commercially viable instant and other extruded products is poised to enter the markets, and is a safe and convenient food vehicle for fortification, hence generating interest from governments and food-aid programs. Other food technologies, such as "economic couscous" in Senegal seems ready to be commercialized by processors.

The biggest "technology" developed by this project in Phase I has been the Hub-and-Spoke Food Processing Innovation System that has shown that rural markets in Africa for locally processed food products exist and can grow, thereby generating rural incomes, empowering women (and youth), and can be used to spread the consumption of nutritionally-fortified foods in rural areas. As described above, we see that the Hub-and-Spoke system can be scaled-up, both locally and in other regions.

B. Intermediate outputs

Because many of the more technology-based new millet and sorghum-based food products are not yet sold in the stores, these could be considered as intermediate outputs. Our consumer and market data (in Niger) indicate that they are near to ready for processors to bring to the commercial market.

7 - STATEMENT ON PROPOSED FUTURE ACTIVITIES FOLLOWING PHASE I RESEARCH ACCOMPLISHMENTS

A. Statement on future activities in Phase II

Niger

In Phase II, we plan to work to scale-up the Hub-and-Spoke Food Processing Innovation System in Niger, and possibly with partner funding in other countries. By "scale-up", in the case of Niger, we mean to expand 1) the individual rural Spokes by making the current and established rural processing innovation centers have the additional role of becoming training centers for secondary and even tertiary Spokes, and 2) to create one or two food technology sub-Hubs in other regions (e.g. Maradi, Zinder) to support and expand the Spoke system further into rural areas and to foster rural markets (Figure 6). This will be done where possible in partnership with the McKnight Foundation project, which we are currently proposing a next phase. Activities will continue in working with urban women processors in Niamey, and perhaps other urban centers, to broaden their product lines, increase scale of operations, and mechanize units. If positive results are obtained from the current market study on extruded instant millet products (instant flours for thick porridges of millet and sorghum, and millet "*fura*"), packaging and market research will be done and efforts will be made to disseminate these processing technologies to urban processors. Product R&D will be conducted to make new and better processed products that are tested regarding market potential. We will work in partnership with the market economics group of SMIL to understand better how to make products that have market potential and can drive businesses. We are additionally considering to pilot an urban and/or rural extrusion facility that would be "shared" by entrepreneurs (or women's associations), where there would be a fee-for-use requirement. Sustainability aspects of the Hub-and-Spoke model will be incorporated into a Phase II proposal, including an increased fee-for-use of the Hub facility for certain functions it serves. We would also like to find assistance to study improvement in social capital, women and youth empowerment, and food security in rural Spoke communities.

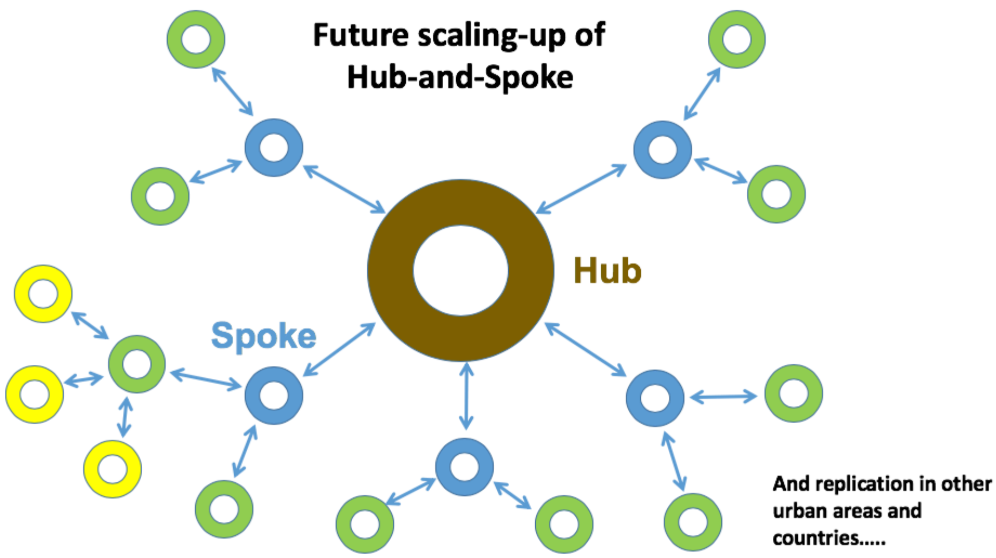


Figure 6. Vision of scale-up of the Hub-and-Spoke Food Processing Innovation model for Niger, and other countries.

<u>*Senegal*</u>

In Phase II, we propose 2 different sets of activities. The first will be complementary to the FPL project to strengthen packaging, labeling, and marketing of extruded instant and instant fortified flours for thin and thick porridges. In Senegal, we have been in talks over the last year with the government (Prime Minister's office, agricultural advisor; office responsible for malnutrition program; Ministry of Industry) regarding scaling-up of our program for entrepreneurial processing of fortified instant flours, and as well with the World Food Programme and a few NGO's working in this area (e.g. CLUSA). A concept note has been requested and one submitted, and a proposal has been written to be submitted to the African Development Bank. Minimally, our plan in the next phase of FPL, and in concert with SMIL, will be to expand capacity to produce fortified instant millet flours through working with our partner processor in Touba, Mme. Mbacke, through training of other entrepreneur processors and introducing more low-cost extruders. This will include traditional fortification as well as application of knowledge on natural fortification strategies gained from Phase I. At a larger level, with partner financing, the vision is to support and expand fortified cereal processing in 3-4 different regions of Senegal. SMIL's role will be to strengthen the marketing side of the project, which has been a weakness of the project.

The second proposed activity will be to expand current work with Dakar (and perhaps in 1 or 2 other urban areas) entrepreneur processors to process high quality competitive millet (and perhaps sorghum) products that consumers want to buy. Convenient millet-based products have been recently improved by ITA through SMIL, such as fermented "economic couscous", instant arraw, and high-quality whole grain instant flours, and the project will work with urban entrepreneurs to help them commercialize them.

<u>*Mali*</u>

As something to think about, we would be interested in starting a small (or medium-sized) project to refashion their existing incubation center at IER/Sotuba (Bamako), which we built and equipped around 2010 in a USAID Mali Mission-funded project, to the current Hub-and-Spoke model. Initial "Spoke" entrepreneurs could be drawn from those we previously worked with, and IER scientists/technologists also on the former project (and one who received her Ph.D. at Purdue with B. Hamaker) would be in the Hub. Technical and organizational support would come from regional SMIL PI's, M. Moussa at INRAN and C. N'Diaye at ITA, to get the Hub-and-

Spoke System working in Mali. We have concurrently suggested in a Concept Note to the McKnight Foundation that one Spoke processing center be funded and to complement production activities in Mali.

Nutrition
There will additionally be a somewhat greater emphasis in nutrition research in the SMIL Phase II for this project. Activities are proposed for two topics:

1. In partnership with McKnight Foundation in Niger, to test the hypothesis that fortified millet or sorghum foods formulated for local rural populations will have better adoption than food-aid blends distributed at rural health clinics, because they are made to the palates of local children, the local women processor association makes the products, and access of the fortified foods to more people is better in rural markets than local health centers. The nutritional target for our projects is to meet 25% of the Recommended Dietary Allowance (RDA) for shortfall micronutrients iron, zinc, and/or pro-vitamin A, and in cases supplementation with a local protein-rich food. Phase II efforts will also need to focus on development/assessment of the value chain for select nutrient dense plant materials (e.g. baobab, moringa, carrot and mango powders). This is a critical component for eventual translation of these technologies.
2. The satiating quality of millet and sorghum foods was studied in Phase I at Purdue, and papers will be published soon on the filling and sustaining quality of these foods to the body (further supporting and expanding on our recently published paper from the INTSORMIL/SMIL projects Cisse et al. Traditional Malian solid foods made from sorghum and millet have markedly slower gastric emptying than rice, potato, or pasta. Nutrients 10:124, 2018). This health-related attribute of millet and sorghum foods could be used as a consumer cue for purchasing and further could be used to promote fortified foods in the market.

B. Linkage to Phase I objectives and activities

The proposed activities above are all extensions of Phase I objectives and activities, including scaling-up of the Hub-and-Spoke Food Processing Innovation System, supporting urban and rural entrepreneurs with locally new technologies and improving food formulations, expanding naturally-fortified instant and non-instant foods and making these foods attractive to be bought by consumers ("market-led nutrition").

C. Training and outreach objectives

There will be further graduate training in the US for at least 2 food scientists and 1 student in the business/market area (one identified in Senegal). Training of entrepreneurs and women processing groups is essential to the project and will be a major component of the projects. Some short-term training may also be included for technical and business-related personnel. This project is largely outreach based (i.e. the concept of the Hub-and-Spoke model). It might be desirable to have a business graduate help to promote the scaling-up of the project to potential donors.

KANSAS STATE UNIVERSITY – AGRICULTURAL ECONOMICS LONG-TERM TRAINING AND RESEARCH

1 – MAJOR ADVISOR

Timothy J. Dalton – *Kansas State University, USA*

2 – TRAINEES

Ph.D.: Tebila Nakelse – *Burkina Faso*

Masters: Mengistu Kassie – *Ethiopia*

3 – PROGRAM OVERVIEW

The College of Agriculture at Kansas State University committed tuition, fees and stipends for two graduate students as part of the cost share contribution towards the Sorghum and Millet Innovation Lab. The two graduate students in the Department of Agricultural Economics are linked to programmatic activities in West Africa and Ethiopia.

West Africa

In West Africa, graduate Tebila Nakelse is studying the impact of price shocks on farmer and consumer welfare in West Africa and consumer demand for new value-added food products developed in the Hamaker project in Niger. In the first study, Mr. Nakelse determined that the food price shocks, and specifically price increases, generate positive welfare effects for rural households and this is dependent upon whether the household is a net seller or buyer of the crop and the degree to which the international price of the commodity is translated to the local price. The causal relationship between world and domestic cereal prices was established using an error correction framework which allowed us to measure and separate long- and short-run effects on domestic price from an exogenous change in world price. Price transmission was higher to producer prices than to consumer prices due to asymmetry in behavioral responses by farmers, middlemen and consumers. Overall, price increases, such as those experienced in the 2008-2009 periods, were associated with an improvement in rural farmers' welfare ranging from 0.02 percent to 0.06 percent. While the magnitude of the net effects are low, they are nonetheless positive and can be associated with poverty reduction to rural households (Table 1).

Table 1: Change in welfare relative to total purchase per commodity and year

	2006	2007	2008	2009	2010	2011	2012	2013	2014
World Price Increase (ζ_{p^w})	19	63	132	70	79	141	136	119	92
Millet	0.08	0.27	0.56	0.30	0.34	0.60	0.58	0.51	0.39
Maize	0.12	0.39	0.81	0.43	0.49	0.86	0.83	0.73	0.56
Rice	0.00	-0.01	-0.03	-0.02	-0.02	-0.03	-0.03	-0.03	-0.02
Sorghum	-0.04	-0.13	-0.28	-0.15	-0.17	-0.30	-0.29	-0.25	-0.20
Total	0.01	0.03	0.05	0.03	0.03	0.06	0.05	0.05	0.04

Stronger integration into world markets, reduced trade barriers, and transaction costs will benefit a country by allowing it and its producers to capture the gains from trade based on comparative advantage as well as the reduced cost of doing business. Public policies and investments that strengthen market incentives and activity, such as improving physical infrastructure, can thereby pay dividends. Although greater integration into world markets will make consumers more vulnerable to fluctuations in world prices, targeted compensation is a preferred policy response, rather than market-distorting policy intervention.

Mr. Nakelse also developed a line of research to determine consumer demand for food product attributes. Non-diversified and poor-quality diets are some of the main causes of hidden hunger and associated illnesses. Yet there is limited research on consumer valuation of food quality attributes especially those related to nutritional quality in low-income countries. Consequently, we assess urban consumers' preference for food quality attributes of value-added cereal products in Niamey, Niger. We combine qualitative and quantitative methods to assess 205 randomly sampled consumers' preferences and the willingness-to-pay (WTP) for food quality attributes. Multinomial logit models are estimated using Maximum Simulated Likelihood and comparing two alterative specifications of consumer preferences. In addition, we account for taste and preference heterogeneity inherent to consumers' responses to a change in quality attributes.

Table 2: Marginal Willingness to pay for all consumers, by gender and income classes, in FCFA using utility model in WTP space

	Observation	Expiration Date	Micronutrient (25 % of DR)	Origin	Family Image
All Classes	4272	242.6***	63.60***	138.3***	41.07**
		(17.81)	(8.428)	(16.61)	(18.60)
Gender					
Male	2,520	203.4***	62.24***	147.8***	44.53*
		(19.05)	(10.39)	(20.01)	(23.52)
Female	1,752	317.4***	70.82***	132.4***	49.69
		(40.31)	(16.17)	(28.66)	(35.92)
Income class ('000 of FCFA)					
Less than 60	888	297.6***	32.40*	145.2***	66.29
		(40.51)	(17.63)	(33.89)	(44.67)
60-120	1368	226.2***	55.05***	203.3***	92.84*
		(46.50)	(19.22)	(45.11)	(51.36)
More than 120	2,016	225.2***	84.89***	104.0***	30.75
		(24.08)	(12.17)	(22.20)	(24.07)
Age					
Less than 28 years old	1,704	249.8***	41.08***	114.7***	28.05
		(21.71)	(10.54)	(20.46)	(23.10)
28-38 years. Old	1,104	221.9***	89.24***	151.4***	77.59**
		(18.38)	(10.78)	(30.60)	(35.44)
More than 38 years. Old	1,464	266.7***	51.34***	185.1***	44.61
		(35.42)	(15.64)	(32.04)	(40.10)

Our study, focusing on urban consumers in one of the poorest nations in the world, yields findings in line with economic theory. Indeed, the hypothetical market intends to provide information on a range on food attributes specifically or broadly related to health, nutrition and country of origin (Table 2). The design of our experiment provides signals to consumers on product freshness, as described by the date of expiration, nutritional content, as described by the micronutrient density, and the origin of the product. In the presence of these treatments, consumers showed strong preference for the quality attributes compared to the situations where they are uncertain on attributes. Specifically, we inferred consumer demand for product safety through a statistically significant WTP for a product that bears a date of expiration compared to a product that does not have such information. In addition, consumers have a strong preference for products that clearly indicate being made in their home country opposed to a situation of uncertainty on the origin. Furthermore, consumers are willing to pay a price premium for given levels of iron. These results are revealing how consumers may react when they are certain about the information given to them when making their food choices. The reduction of uncertainty drives higher willingness to pay for product related attributes.

Our attributes, and the information given to participants grounded or enhanced those beliefs. In addition, the effectiveness of how information is given to consumers has been crucial on consumers' WTP. For examples, the low level of micronutrient WTP may be related to consumer-limited knowledge on its health benefit. The date of expiration or product origin is widely understood by consumers as well as straightforwardly explained, which is not the case of micronutrients. Hence, participants may be uncertain on the associated health benefit, which makes information less effective in reducing consumer's health outcome variance. Aside from these results, findings are heterogeneous about some socioeconomic characteristics, such as gender and consumer's income class, that segments market classes.

Because market demand is high for health related attributes such as date of expiration, public policy could be undertaken to create an enabling environment for supply of such attributes. This could be done by first taking appropriate measures on sensitizing food processors on the economic benefit that could be generated by following quality standards and clearly informing consumers on those attributes. This information could be provided to consumers using harmonized and high quality labels and packaging. This high quality label is critical in giving consumers trust on the claimed attributes. Furthermore, government could set some guidelines on the type of label that should be adopted by food processors. Such guidelines are already adopted by several countries in West Africa on cigarettes following FAO and WHO Codex Alimentarus Commission standards.

In addition, our study suggests that micronutrient demand is higher in wealthier income class and for women. The income distribution implication of goods is important since the growth in demand for health care and a food system which allocates more resources to supply health or nutrition enhancing attributes, can create a situation where low-income households are priced out of the market despite their health and nutrition vulnerability. This finding can be counterbalanced with the fact that demand for micronutrients is greater in women groups and suggests that targeted policy intervention to alleviate hidden hunger or food quality deficiency may be effective.

Even though our results suggest possible micronutrient demand, especially for women and higher income class, additional policies are necessary to move forward the fight against malnutrition and micronutrient deficiency. This is so because the poor, who are really in need of such micronutrients, are less willing to pay for it mainly because of a resource constraints or the lack of knowledge on short- or long- term health benefits. In addition, since the aggregate health outcome of those attributes is a public good, it is likely that market provision of it would not be Pareto Optimal. Government intervention therefore may be necessary to achieve optimal provision of those attributes. Such intervention could occur by targeting the most in need: women in low income classes. Nevertheless, a proper targeting of these group of consumers could be costly for the entire population especially when it is based on income. The transaction costs associated with this targeting could increase the cost of the program and the tax burden. Instead of income targeting, gender targeting seems easier to implement and could have the highest return per dollar invested because pregnant and women are the most in need of micronutrients, especially iron.

Finally our study found that consumers are likely to be ethnocentric in food products, especially younger consumers. This consistent with findings in other studies on consumers preference for a product image of country of origin. One explanation of this result is that with increased globalization and economic development, consumers developing countries may have realized that locally produced products are becoming increasingly competitive, if not yet equal, to imported products. This is important for food processors from both developed and developing countries because they have the opportunity across such countries to exploit, and to support, the country associated with their companies. Such research therefore remains of significant relevance to international businesses and local entrepreneurs.

Ethiopia

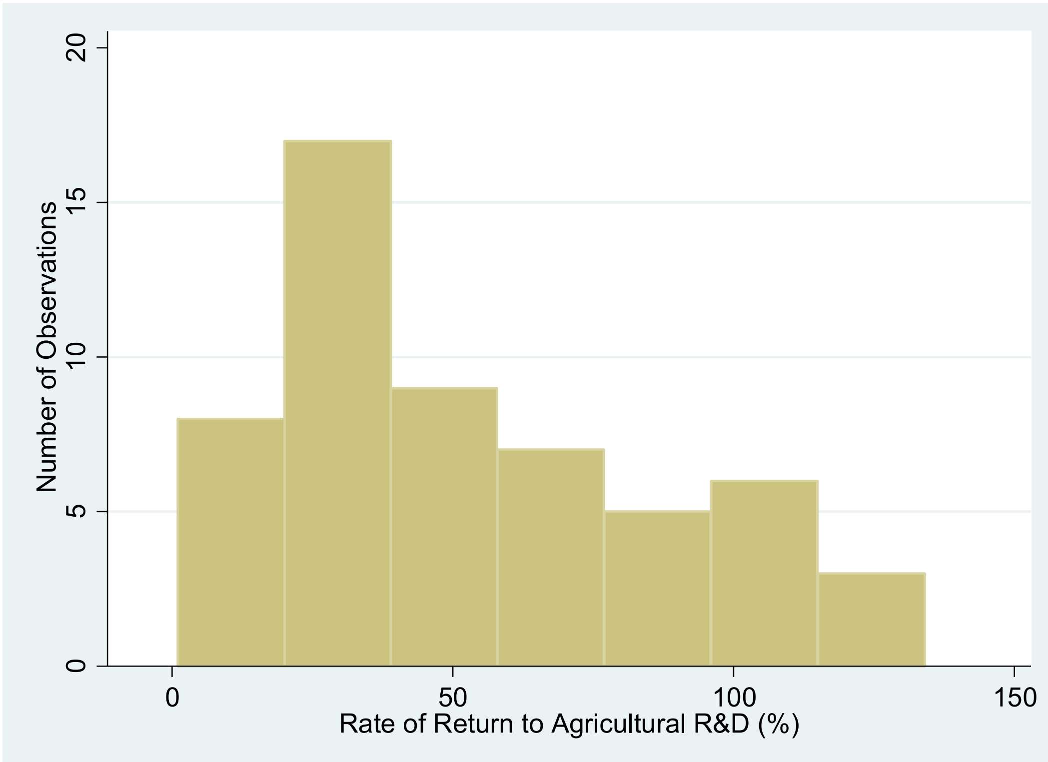
Mr. Mengistu Kassie is pursuing an M.S. degree and is developing an equilibrium displacement model on the impact of technologies to control sorghum yield loss associated with Striga. This research is in an early stage and will be completed in 2019. Mr. Mengistu is disaggregating sorghum production in Ethiopia by region and retaining population representation through utilization of the World Bank Living Standards Management Survey Integrated Surveys in Agriculture. Potential impacts from technological innovations, namely seed and integrated Striga management packages, are drawn from the secondary literature. One weakness in the analysis is that there does not exist a widespread literature on the field level impacts of these innovations and a paucity of information on research station trials.

Meta-analysis of the Returns to Sorghum Research and Development

In 2017, Yacob Zereyesus and Dalton published a study on the actors affecting the internal rate of return to sorghum and pearl millet research and development. Sorghum and millet grow in some of the most heterogeneous and austere agroecologies around the world. These two crops are amongst the top sources of food and feed crops and their significance will continue to grow especially with climate-related heat and drought stress. Yet, few studies document the impact of sorghum and millet genetic enhancement. The Internal Rate of Return (ROR) is one of the most popular metrics used to measure the economic return on investment on agricultural research and development (R&D). The study conducted a meta-analysis of 59 sorghum and millet estimates. The average rate of return to sorghum and millet R&D investment is in the range of 54-76 percent per year (Figure 1).

All of the reviewed studies computed social rather than private ROR because sorghum and millet technologies were developed using public funds originating from host country National Agricultural Research Systems (NARS) and international organizations such as the INTSORMIL CRSP, ICRISAT and others. Nearly three quarter of the studies focused only on sorghum (72 percent) and around one tenth of the studies (8 percent) dealt only with millet. Regression models are used to analyze the determinants of variations in the reported RORs. The ROR measure characteristics, analysist characteristics, research characteristics, and evaluation characteristics are used as control variables. Results show that ex-ante type and self-evaluated type of analyses are positively and significantly associated with the ROR estimates. Compared to estimates conducted by a university, results from international institutions and other mixed organizations provided significantly smaller estimates. Estimates conducted at national level also are significantly lower than those conducted at sub-national levels. The study also reconstructed modified internal rate of return (MIRR) for a sub-sample of the reported RORs following recent methods from the literature. These results show that the MIRR estimates are significantly smaller than the reported ROR estimates.

Figure 1: Distributions of Rates of Return to Agricultural R&D for Sorghum and Millet excluding the extreme values



Average rate of return to agricultural R&D = 54.0, standard deviation = 37.0

Historical returns on sorghum and millet R &D investments have been socially profitable. On a global coverage, the average ROR to sorghum and millet agricultural R&D investments is in the range of 58-81 percent per year. When millet studies are excluded, the average rate of return for sorghum was about 97.6 percent per year. A number of notable results are observed from the review. For example, the host country national agricultural research systems (NARS) and international partners such as the International Sorghum and Millet Collaborative Research Support Program (INTSORMIL CRSP), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and other collaborating institutions have played a major role in supplying sorghum and millet technologies, and most frequently improved. The majority of the economic impact assessment studies are evaluations of past R&D investments (i.e. ex-post type analyses), especially in the sub-Saharan African countries. For sorghum, the systematic influence of the analyst characteristics (published-or-not and self-evaluated-or-not) is important. For example, self-evaluated sorghum impact assessment studies appear to have higher RORs.

Investment rate of return estimates systematically overstate the returns to agricultural R&D. The study also reconstructed modified internal rate of return (MIRR) for a sub-sample of the reported RORs following recent methods from the literature. These results show that the MIRR estimates are considerably smaller than the reported ROR estimates. It is important to note that the limited number of reported ROR estimates limits the generalization of the regression results of the study. The lack of variation in some of the determinants of the RORs also resulted in exclusion of some important variables.

Publications

Journal Articles

Nakelse, Tebila, T.J. Dalton, N. Hendricks, H. Manzamasso (2018), "Are Smallholder Farmers Better or Worse Off from an Increase in the International Price of Cereals?". Food Policy 79 (2018) 213–223.

Nakelse, T. (2018). Are Urban Consumers in Niger Willing to Pay for Safe and Nutritious Food? Submitted.

Zereyesus Y.A., Dalton T.J. 2017. Rates of return to sorghum and millet research investments: A meta-analysis. PLoS ONE 12(7): e0180414. https://doi.org/10.1371/journal.pone.0180414.

List of Presentations

Tebila Nakelse, Timothy Dalton, Nathan Hendricks, Hodjo Manzamasso (2018), Impact of World Cereal Price Change on Rural Household Welfare in Burkina Faso Oxford University Centre for the Study of African Economies Conference, March 18-20, Oxford, United Kingdom.

Tebila Nakelse, Timothy Dalton and Yaye Hama (2018) Are Urban Consumers Willing to pay for Safer and Nutritious Food in Niger? The 22th International Consortium on Applied Bio-economy Research (ICABR) Conference, June 12-15, Washington DC, USA

Tebila Nakelse, Timothy Dalton and Yaye Hama, Are Urban Consumers Willing to pay for Safer and Nutritious Food in Niger? 30th International Conference of Agricultural Economists (ICAE), July 28-August2, Vancouver, British Columbia, Canada.

Tebila Nakelse, Timothy Dalton and Yaye Hama, Are Urban Consumers Willing to pay for Safer and Nutritious Food in Niger? Sorghum in the 21st Century, April 9-12, Cape Town, South Africa.

Tebila Nakelse, Timothy Dalton and Yaye Hama (2017), "Cost and Return Analysis of Cereal Value Added Food Processing Sector in Niger", March 6-9 SMIL Annual Review Meetings - Mbour, Senegal.

Tebila Nakelse, Timothy Dalton and Yaye Hama (2017), "Cost and Return Analysis of Cereal Value Added Food Processing Sector in Niger" 2017 SMIL Project Actor in Niger Meeting, May 24, Niamey, Niger.

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