SMIL Annual Report

Genetic Improvement of Sorghum for Resistance to Fungal Pathogens
Aims

• Development of disease resistant, improved varieties
• Genetic and genomic studies to identify resistance genes, define resistance loci
• Core collection
• Training
Area of Research

- Pathology
- Molecular biology
- Resistance breeding
- Genetics and genomics

❖ Project contains activities with immediate to long-term impacts
Outline

• Premise of project
• Breeding activities
• Released varieties
• Seed increases, popularization, and demonstrations
• Mapping of new fungal resistance genes
• Characterization of Ethiopia core collection
• Building human capacity
• Future directions
Purdue-Mengiste Lab
- Genetic and genomic characterization of a core collection of Ethiopian germplasm
- Genetic screen for resistance-exploring the natural variation in sorghum
- Anthracnose and grain mold resistance genes
- Training

EIAR, & Regional centers conduct nurseries and trials
- Breeding varieties for adaptation, high yield, and disease resistance
- Conduct variety verifications for variety release, demonstrations, and seed increases
- Phenotyping of the core collection and other germplasm
- Pathology: studies on fungal strains

Collaborations: Bako, Jimma, Asosa, Haramaya, Purdue
Trial sites

Pawe Research Center
Assosa Research Center
Jimma Research Center
I. Developing sorghum varieties with resistance to diseases, improved yield and broad adaptation

• Diseases nurseries and trials
• Direct selections
• Introgression of resistance genes into elite materials or released varieties
1. Introgression of disease resistance into adapted and improved varieties
   - Crosses between resistant materials and elites or released varieties
   - Evaluation of segregating populations at different stages
2. Preliminary Yield Trials for grain mold resistance: resulting from crosses
3. Observation nursery trial of selected sorghum landraces for disease resistance
   - To be advanced to PYT
4. Evaluation of new RILs (selected lines will be advanced to PYT)
5. Characterization of the Ethiopian germplasm (SMIL core collection)
   - Seed increase at MARC and trial at Haramaya (final year)
6. Seed increases and promotion of new materials
Major achievements: Merera

- Merera was officially released in 2020
- Major achievement of SMIL project
- Yield advantage = 43 %
- Yield potential ~ 5.4 tones ha\(^{-1}\)
- Bird tolerant
- Stay green; dual purpose (food and forage)

• Merera triggered many collaborations
Jabaa

- Released in 2022
- Yield potential: 4.2 ton ha$^{-1}$
- Yield advantage: 39.1%
- Early variety: better adapted to erratic rain fall
- Short stalk: tolerant to lodging
- Resistant to both foliar and panicle diseases
- Resistant to bird attack
- Seed color is preferred for consumption and market

‘Jabaa’ variety field performance at dough stage
Beside demonstration activity, we conducted seed multiplication, 2021

10 tons of seed harvested and stored properly

For further scaling up of the variety
Demonstration of ‘Merera’…

- Training of farmers, developmental agents…..
- Demonstration activities
- Preparation of manuals, leaflets on production and management practices
- Potential ‘kebeles’ from each district consisting Farmers Research and Extension Group (FREG) unit comprising of farmers were established
- Gender and youth balance in each FREG unit
Field days

- Farmers
- Seed enterprises
- Woreda expertise & DA’s
- Investors
- Other stake holders

Media

- 6 - national and regional

https://youtu.be/AUg0fLPSFSY
https://youtu.be/muOdEpSdH2M

Field days organized by Bako Ag. Research Center, 25-28 October 2021.

<table>
<thead>
<tr>
<th>No</th>
<th>Field days</th>
<th>Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Farmers</td>
</tr>
<tr>
<td>1</td>
<td>Demonstration</td>
<td>M F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Seed multiplication</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
</tr>
</tbody>
</table>

Grand total 273
Merera seed multiplication and scaling out activities, 2022

<table>
<thead>
<tr>
<th>Activities</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scaling out activities</strong></td>
<td></td>
</tr>
<tr>
<td>• Large-scale scaling out activities</td>
<td>85 farmers</td>
</tr>
<tr>
<td>• Small seed packs distribution</td>
<td>100 farmers</td>
</tr>
<tr>
<td>• Cluster-based scaling out activities</td>
<td>14 farmers</td>
</tr>
<tr>
<td>Investors involved in scaling activities</td>
<td>2</td>
</tr>
<tr>
<td><strong>Amount of seed dispatched</strong></td>
<td>4 ton</td>
</tr>
<tr>
<td><strong>Area covered by improved seed (total)</strong></td>
<td>333 ha</td>
</tr>
<tr>
<td><strong>Seed multiplication</strong></td>
<td>0.5 ha</td>
</tr>
</tbody>
</table>

- Activities were limited due to security in the area
Genetics and genomics of disease resistance and Ethiopian germplasm

- Genetic screens for disease resistant materials
- Map and/or identify resistance genes
  - Biparental mapping
  - Genome wide association studies
- Characterization of the Ethiopian SMIL-core land race collection
- New experimental population for breeding and genetic mapping
### Anthracnose resistance genes

<table>
<thead>
<tr>
<th>Genes or Genotypes</th>
<th>Fungal strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Csgl._1</td>
</tr>
<tr>
<td>ARG1</td>
<td>R</td>
</tr>
<tr>
<td>ARG2</td>
<td>R</td>
</tr>
<tr>
<td>ARG3</td>
<td>S</td>
</tr>
<tr>
<td>ARG4</td>
<td>R</td>
</tr>
<tr>
<td>ARG5</td>
<td>R</td>
</tr>
<tr>
<td>PML981442</td>
<td>R</td>
</tr>
<tr>
<td>PML981475</td>
<td>R</td>
</tr>
<tr>
<td>PML981476</td>
<td>R</td>
</tr>
<tr>
<td>PML981488</td>
<td>R</td>
</tr>
<tr>
<td>TAM428</td>
<td>S</td>
</tr>
<tr>
<td>BTx623</td>
<td>S</td>
</tr>
</tbody>
</table>

- ARG1 and ARG2 are NLR genes
- ARG3, fine mapping complete, validation
- ARG4 and ARG5, NLRs, complete
- Mapping for ARG 6-9 is underway
- New genes from the SMIL core
- Resistance to grain and leaf diseases in the PMLs
### Multi-pathogen resistance lines

#### Anthracnose  Grain mold

<table>
<thead>
<tr>
<th>Line</th>
<th>Anthracnose</th>
<th>Grain mold</th>
</tr>
</thead>
<tbody>
<tr>
<td>PML981476</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>PML981475</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>PML981488</td>
<td>R, broad</td>
<td>R</td>
</tr>
<tr>
<td>PML981299</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>PML981442</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>PML981446</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>PML981472</td>
<td>R, broad</td>
<td>R</td>
</tr>
<tr>
<td>PML981474</td>
<td>R, broad</td>
<td>R</td>
</tr>
<tr>
<td>SAP135</td>
<td>R, broad</td>
<td>ND</td>
</tr>
<tr>
<td>SC283</td>
<td>R, broad</td>
<td>ND</td>
</tr>
</tbody>
</table>

Bako disease nursery
“One gene closer to a superman sorghum”

As climate change events shift or necessitate the production of dryland crops such as sorghum into higher rainfall or irrigated regions, leaf diseases become even more significant,” “It is precisely in those situations where powerful genes become so crucially important.” (Gebisa Ejeta)
Gene specific molecular markers
**Hyphal growth**

**SUMMARY**

Sorghum is an important food and feed crop globally. Its production is hampered by anthracnose disease caused by the fungal pathogen Colletotrichum sublineola (Cul). Here, we report identification and characterization of ANTHRACNOSE RESISTANCE GENE2 (ARG2), encoding a nucleotide-binding leucine-rich repeat (NLR) protein that confers race-specific resistance to Cs strains. ARG2 is one of a cluster of several NLR genes initially identified in the sorghum differential line SC328C that is resistant to some Cs strains. This cluster shows structural and copy number variations in different sorghum genotypes. Different sorghum lines carrying independent ARG2 alleles provided the genetic validation for the identity of the ARG2 gene. ARG2 expression is induced by Cs and chitin induces ARG2 expression in resistant but not in susceptible lines. ARG2-mediated resistance is accompanied by higher expression of defense and secondary metabolite genes at early stages of infection, and anthocyanin and zeaxanthin metabolites are upregulated in resistant plants. Interestingly, ARG2 localizes to the plasma membrane when transiently expressed in Nicotiana benthamiana. Importantly, ARG2 plants produced higher shoot dry matter than non-transformed lines carrying the susceptible allele suggesting an absence of an ARG2-associated growth trade-off. Furthermore, ARG2-mediated resistance is stable at a wide range of temperatures. Our observations open avenues for resistance breeding and for dissecting mechanisms of resistance.
Synteny between *S. bicolor* and *O. sativa* at the ARG3 locus

Mapping using resistance to Cs29, Csgrg
Recombination analysis across candidate genomic region harboring ARG6

<table>
<thead>
<tr>
<th>Chr.08</th>
<th>Markers position (Mbp)</th>
<th>Recombination frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.16</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>0.54</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>0.62</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>0.79</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>0.97</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td>4.50</td>
<td>5.00</td>
</tr>
</tbody>
</table>

InDel

- -57 177(5)
- -11 177(1)
- -20 177(0)
- -13 177(0)
- -26 177(0)
- -64 177(0)
- -67 177(2)
- -29 177(3)
- -31 177(5)
- -32 177(12)
- -35 177(19)
- -38 177(28)
- -41 177(49)
- -43 177(62)
- -45 177(67)
- -47 177(78)

PML981475
Fine mapping of ARG6 gene
Summary

- Identified 5 major genes, defined or cloned and additional three loci
- Tightly linked or gene specific markers for six genes
- Four NBs-LRR proteins
- ARG3 is protein of unknown function

ARG1, ARG2, ARG4, ARG5

R proteins
NBS-LRR

Canonical R proteins have a leucine-rich repeat
Why does this matter?

• Opens avenues for molecular breeding and application of other genetic technologies

• Tightly linked or gene specific molecular markers

• Stacking genes for broader resistance
Ethiopian Sorghum Germplasm Characterization

- Assess diversity and population structure among landrace and cultivars.
- Discovery /mining of loci/genes underlying important traits.
- Development of a core subset (SMIL-Core).
- Facilitate utilization of germplasm for breeding, genetics and evolutionary studies.
### Phenotypic descriptors used to characterize the germplasm collection

<table>
<thead>
<tr>
<th>Quantitative Traits</th>
<th>Categorical Traits</th>
<th>Response to disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Days to 50% flowering</td>
<td>- Seed form</td>
<td>- Resistance to grain mold</td>
</tr>
<tr>
<td>- Days to maturity</td>
<td>- Leaf mid rib colour</td>
<td>- Resistance to Anthracnose</td>
</tr>
<tr>
<td>- Plant height (cm)</td>
<td>- Stay green</td>
<td>- Resistance to Smut</td>
</tr>
<tr>
<td>- 1000 Kernel weight</td>
<td>- Head exsertion</td>
<td>- Resistance to other foliar disease</td>
</tr>
<tr>
<td>- Grain yield per panicle (g)</td>
<td>- Head compactness and shape</td>
<td></td>
</tr>
<tr>
<td>- Seed number per panicle</td>
<td>- Awns</td>
<td></td>
</tr>
<tr>
<td>- SPAD</td>
<td>- Kernel covering</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Kernel colour</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Kernel plumpness</td>
<td></td>
</tr>
</tbody>
</table>
The genomics component

- About 80% of the collection (>1600) were genotyped by sequencing
- Genetic diversity and population structure
- Association studies/discovery of candidate loci_genes implicated in important traits.
- SMIL-Core selection: More to come here
Almost half of the varieties including inbred lines had ancestry membership coefficient with likelihood of more than 0.90.

Two genotypes (Melkam/Meko/Misikir and Dagim/Birimash/IS9302) represent the genetic backgrounds of almost all cultivars.

Genetic uniformity arising from recycling of parental sources in breeding programs is a bottleneck.

It is important to maximize variability in the breeding parents.
Whole genome resequencing of SMIL core Ethiopian landraces

- DOE/Joint and Genome Institute sequencing 400 Ethiopian sorghum lines (30x minimum)
- The SMIL core and popular landrace varieties
- Foundation for genome enabled breeding
Characterization of SMIL core Ethiopian landraces

• Pathogen resistance and other traits (Chemeda, PhD dissertation)
• Drought tolerance traits (Transpiration efficiency)
• Striga resistance
358 landraces evaluated at Bako, Jimma, Asosa, and at Haramaya

The sites are hot spots for disease, good for screening germplasm, and evaluate yield potential

Scored anthracnose, rust, turcicum leaf blight, gray leaf spot, and grain mold

Yield and agronomic traits
Anthracnose resistance in the sorghum SMIL core

The data from Bako and Jimma (pooled over years in each location)

**Bako**
- 5 accessions are immune/healthy
- 196 accessions showed HR reactions
- 157 genotypes are susceptible
- Heritability (%) = 69

**Jimma**
- 4 accessions are immune/healthy
- 177 accessions showed HR reactions
- 177 accessions are susceptible
- Heritability (%) = 64

❖ **130** (36%) showed consistent resistant reaction across locations
❖ **106** (30%) showed consistent susceptible reaction across locations
❖ **122** (34%) not consistent across locations, **71** accessions resistant Bako, susceptible at Jimma and **51** accessions resistant at Jimma but susceptible at Bako
Some disease reaction before flowering at Bako
GWAS analysis has been conducted on anthracnose, rust, gray leafspot, grain mold and other agronomic traits.

GWAS for anthracnose resistance identified a total of **32 loci** distributed across sorghum chromosomes.

We found also significant loci associated with rust, gray leaf spot and grain mold.

GWAS for 12 agronomic traits identified significant loci that associated with different traits.

*(Based on Data from natural infestation in the field)*
Genome-wide association analysis of anthracnose resistance. Manhattan and QQ plots of association mapping for anthracnose at Bako (a) and Jimma (b) using BLINK.
Green house anthracnose resistance screening of the SMIL-core

- SMIL core and popular landraces
- Simultaneous inoculation with multiple strains (Csgl-1, Csgl-2, Csgl-27, Csgl-29, Csgrg)
- Broad spectrum resistance

Chemeda

Green house screening for anthracnose resistance
Broad spectrum anthracnose resistance-resistance to mixed strains
New resources for breeders and genetic studies

- Seven independent RILs (2100 lines) developed for comprehensive mapping of disease resistance and other traits.
- Integrate into breeding program
- Genetic mapping
- Parental lines combine resistance to leaf and grain diseases, stay green....
New breeding and mapping populations

- Field trials and data capture
- Three RIL populations of >500 planted at Jimma, Assosa, and Bako
- Integrate into breeding (initiated)
- Generate GBS or low pass NGS for 700 RILs (~100 per population)
- Comprehensive mapping of resistance & other traits
- Impact: short to medium term
Evaluation of Recombinant Inbred Lines at Jimma, Bako, and Assosa

- Three RIL populations were planted at 3 locations
- A total of 564 selected lines including the resistant (P9830 and SAP135) and susceptible (TAM428) checks
- 161 recombinant inbred lines showed resistant (scored 1) than checks
- Best performing and resistance lines will be selected and advanced for further evaluation
- Data being analyzed but happy to see some of the centers have clear plans for the utilization these materials
Data for Anthracnose, oval leaf spot, leaf blight and Rust were scored
Human Capacity

• SMIL II- 2 PhDs

• Two short term training at Purdue
  – Chemeda, at Purdue
  – Assefa Gidesa

• Two significant workshops at MARC
PhD dissertation titles

Assefa Gidesa, Ambo University
Distribution, Pathogen Variability and Management of Sorghum Anthracnose (*Colletotrichum sublineola*) in South-Western Regions of Ethiopia

Chemeda Berhanu, Haramaya University
Genetic Diversity, Genotype by Environment Interaction and Genome Wide Association Mapping For Anthracnose (*Colletotrichum sublineola*) Resistance in Ethiopian Sorghum Core Collection

Moges Mekonen, Addis Abeba University
Genetic diversity and association mapping of virulence gene in *Colletotrichum sublineola*
Current mentees

- Gezahegn is a post doc who is still the main driver in the genomic studies and training of others

- Grad students: Chemeda Berhanu and Pascal Okoye, Assefa Gidesa
Future

- Promote released varieties and materials in the pipeline
- Seed production and collaboration on end user traits ….
- Advance materials from crossing towards variety
- Open access publications for ARGs, and team publication on the core
- Multi-location testing for new RILs in Ethiopia and advance selected RILs
- Analyses of the whole genome sequences of the Ethiopian core
- Introgression of ARGs

❖ Next phases- transfer ARGs into elite materials through a locally led effort
Key achievements and impact

- Genomic and phenotypic characterization of the core
- SMIL sorghum cores materials are intensively explored for breeding program
- New variety “Merera” and “Jabaa” were released by Bako ARC
- Materials from crosses advancing through the breeding scheme
- Source of disease resistance genes identified for future breeding
- Multiple materials in pipeline for future release
- Training students
Challenges

- Security issues in Western Ethiopia
- Weaker follow up
- Lack of molecular facilities and other infrastructure
Alemu Tirfessa, EIAR, Melkassa
Alemnesh Bekele, Haramaya University
Assefa Gidesa*, EIAR, Assosa
Chemed Berhanu*, OARI, Bako
Gebisa Ejeta, Purdue
Getachew Ayana, EIAR, Melkassa
Gezahegn Girma, Purdue
Gudeta Bedhadha, OARI, Bako
Habtamu Alemu, EIAR, Assosa
Habte Nida, Purdue
Kebede Dessalegn, OARI, Bako
Moges Mekonen, EIAR, Melkassa
Nasria, EIAR, Jimma
Solomon Admasu, EIAR, Jimma
Tamirat Bejiga, EIAR, Melkassa