2023 ANNUAL REVIEW MEETING

February 2-3, 2023
Genetic Enhancement of Sorghum to Promote Commercial Seed Supply and Grain Market Development

PI: Gebisa Ejeta, Co-PI: Habte Nida
Aim

- Develop a functional sorghum breeding program focused on developing adapted and high-yielding hybrid cultivars
  - Hybrid cultivars tolerate environmental stress and yield higher
  - Hybrid cultivars to strengthen the seed supply value chain and catalyze the development of a commercial sorghum seed enterprise system
Objectives

1) To evaluate Ethiopian sorghum landraces for drought tolerance and Striga resistance to produce phenotypic data for these traits to associate with the sequence data generated in Phase I

2) To establish heterotic pools among Ethiopian sorghum landraces

3) To develop elite parental lines for a sorghum hybrid program from Ethiopian landraces

4) To establish a stepwise synthesis and testing of experimental sorghum hybrids from introduced germplasm and Ethiopian landraces improved for multiple agronomic traits

5) To promote and support the development of a hybrid seed production system in Ethiopia
1. Evaluation of Ethiopian sorghum landraces for drought and Striga

1.1 Field-based drought phenotyping & GWAS

- A core subset of 358 landraces evaluated for drought tolerance using contrasting environments
  - Non-stress irrigated condition – Melkassa
  - Dryland (a stress condition) – Mieso
- Data on drought and agronomic data collected and analyzed
I. Evaluation of Ethiopian sorghum landraces for drought and Striga

1.1 Field-based drought phenotyping & GWAS

QTLs associated with drought tolerance identified

- 3 significant loci detected based on GWAS for stress tolerance index
- 2 of these were also consistently detected for yield under stress condition suggesting they could be major QTLs associated with drought tolerance in the crop
- The nature of the QTLs and landraces that carry the tolerance allele is yet to be determined and validated
QTLs associated with drought stress tolerance

GWAS for drought tolerance: Significant QTLs associated with drought stress tolerance index (STI)
1. Evaluation of Ethiopian sorghum landraces for drought and Striga

1.1 Field-based drought phenotyping & GWAS

- Drought tolerant landraces identified that can be used for breeding
  - 40 landraces showed excellent ratings for drought and agronomic traits and another 70 showed moderate drought tolerance (2021 data)
- Landraces that showed an overall excellent drought tolerance already included in the crossing program and variety development
  - Example: 93 landraces included in the 2022 variety trial (PYT stage)
1. Evaluation of Ethiopian sorghum landraces for drought and Striga

1.2 Controlled drought phenotyping

- Controlled experiments for drought traits: TE and root system architecture (RSA) in good progress at Melkassa and Jimma, respectively.
- A PhD student (Abel) is engaged in this
1. Evaluation of Ethiopian sorghum landraces for drought and Striga

1.3 Screening for the low germination stimulant (lgs) marker

- Over 2000 Ethiopian landraces and working collections from SMIL Phase I were screened for the lgs marker at Purdue University (USA)

- 35 Landraces found to carry the resistant allele (lgs) and these are mostly from Eastern Wellega, Benishangul Gumuz, Illubabor, and Western Hararghe

- Most of the landraces originated in the drylands where Striga is a major threat appeared to produce high germination stimulant – they would be susceptible unless they have a different resistance mechanism
1. Evaluation of Ethiopian sorghum landraces for drought and Striga

1.3 Summary of the *low germination stimulant (lgs)* marker data

<table>
<thead>
<tr>
<th>LGS marker</th>
<th>Control marker</th>
<th>Number of landraces</th>
<th>Expected resistance reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>✓</td>
<td>35</td>
<td>Resistant</td>
</tr>
<tr>
<td>M</td>
<td>-</td>
<td>56</td>
<td>-</td>
</tr>
<tr>
<td>H</td>
<td>✓</td>
<td>839</td>
<td>Susceptible</td>
</tr>
<tr>
<td>H</td>
<td>-</td>
<td>213</td>
<td>Susceptible</td>
</tr>
<tr>
<td>L</td>
<td>✓</td>
<td>573</td>
<td>Susceptible</td>
</tr>
<tr>
<td>H+L</td>
<td>✓</td>
<td>93</td>
<td>Susceptible</td>
</tr>
<tr>
<td>L</td>
<td>-</td>
<td>71</td>
<td>Susceptible</td>
</tr>
<tr>
<td>No sample + others (F, VF)</td>
<td></td>
<td>130</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>2010</strong></td>
<td></td>
</tr>
</tbody>
</table>

LGS1 CODE:
- L=Light like SQR
- H=Very heavy like BTX623
- VF= Very Faint
- F = Faint band
- M=Missing
- ✓=control marker present
I. Evaluation of Ethiopian sorghum landraces for drought and Striga

1.4 Phenotyping for Striga resistance

- Field-based: this has been affected by the conflict in Northern Ethiopia
  - 2020 - the activity was initiated as a PhD thesis (Hailegebriel Kinfe)
    - 200 landraces were screened for field Striga resistance at three locations in Tigray
    - On November 2020 the conflict broke out in the region
  - 2021 – we tried to do the study in Amhara region (MSc and PhD students were engaged)
    - Disrupted by the conflict that expanded to Amhara region starting July 2021
1. Evaluation of Ethiopian sorghum landraces for drought and Striga

1.4 Phenotyping for Striga resistance

- Phenotyping in a controlled environment:
- A PhD student (Abiy) phenotyping the 358 core subset of landraces for Striga

A controlled Striga research facility at Holeta, Ethiopia
2. Establish heterotic pools among Ethiopian sorghum landraces

**Background**

- Sorghum germplasm generally classified into two major parental groups
  - Seed parents (A/B)
  - Pollinator parent (R)
- Currently the two groups serve as *de facto* heterotic groups for sorghum
- The heterotic responses among germplasm within each of these two parental gene pools have not been investigated
- This activity will give us evidence if greater heterosis can be achieved within each parental pool and will facilitate the selection of landraces for conversion
2. Establish heterotic pools among Ethiopian sorghum landraces

**Background**

- Sterility reaction of over 1300 landraces and working collections studied during phase I
- Unique clustering pattern and geographic adaptation (manuscript in prep)

**Heterotic pool and combining ability study in phase II**

- A selection of 100 landraces (widely grown by farmers and representing all agro-ecologies) used in heterotic pool and combining ability study
  - 60 Landraces with R reaction and
  - 40 landraces with B reaction

\[
\text{X} \quad 6 \text{ testers (3 A-lines and 3 R-lines)}
\]
2. Establish heterotic pools among Ethiopian sorghum landraces

Hybrids of local landraces tested in the past three years

<table>
<thead>
<tr>
<th>Year</th>
<th>Hybrids</th>
<th>Parental lines</th>
<th>Checks</th>
<th>Total entries</th>
<th>Reps &amp; Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td>2020</td>
<td>108</td>
<td>56</td>
<td>5</td>
<td>169</td>
<td>2 reps + 2 locations</td>
</tr>
<tr>
<td>2021</td>
<td>309</td>
<td>60</td>
<td>3</td>
<td>372</td>
<td>2 reps + 1 location</td>
</tr>
<tr>
<td>2022</td>
<td>510</td>
<td>101</td>
<td>3</td>
<td>614</td>
<td>2 reps + 2 locations</td>
</tr>
<tr>
<td>Total</td>
<td>927</td>
<td>217</td>
<td>11</td>
<td>1155</td>
<td></td>
</tr>
</tbody>
</table>
2. Establish heterotic pools among Ethiopian sorghum landraces

Yield potential of hybrid combinations

- Landraces that can make the best hybrids identified based on the previous two years of data
- Data from the 2022 field test is not yet ready for analysis
- Final data analysis and recommendations will be made upon completion of the 2022 data processing

Yield potential of different hybrid combinations (2021 year data)
3. Parental lines development for hybrid program
3. Parental lines development for hybrid program

Development of landrace B-lines

- Conversion of selected landraces into sterile versions to be used as female parents progressed very well

- Currently at the third cycle of conversion/sterilization, 219 A/B pairs of landraces have been generated and planted in the off-season (2022-2023)

- After about 2 more cycles of sterilization/conversion (i.e. within one and a half years), they can be ready for hybrid synthesis
3. Parental lines development for hybrid program

Development of landrace R-lines

- From the 2022 field test, 147 R by R populations of landraces advanced into F3 via SSD approach
- The population planted in the off-season (2023) as generation advance
- By the end of the year, we should have a set of R-line versions of the landraces at an advanced stage that can be used in the synthesis of the first generation of hybrids based on native germplasm

R by R population, Mieso 2022
4. Stepwise synthesis and testing of experimental hybrids

Hybrids synthesized at Purdue – multi environment test

1. Dual purpose hybrids
2. Drought tolerant hybrids
## Dual purpose hybrids

Summary of yield and injera making quality of candidate dual hybrids (2017-2021)

<table>
<thead>
<tr>
<th>No</th>
<th>Hybrids</th>
<th>Grain yield – all environment (t/ha)</th>
<th>Dry Biomass (t/ha)</th>
<th>Injera consumer preference (1-9 scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>K10538</td>
<td>4.5</td>
<td>9.9</td>
<td>6.5</td>
</tr>
<tr>
<td>2</td>
<td>K10541</td>
<td>5.2</td>
<td>8.5</td>
<td>7.0</td>
</tr>
<tr>
<td>3</td>
<td>K10544</td>
<td>4.7</td>
<td>8.2</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>K10550</td>
<td>4.7</td>
<td>9.1</td>
<td>5.0</td>
</tr>
<tr>
<td>5</td>
<td>K10552</td>
<td>4.8</td>
<td>8.7</td>
<td>7.0</td>
</tr>
<tr>
<td>6</td>
<td>ESH5 (Check)</td>
<td>4.9</td>
<td>7.2</td>
<td>7.5</td>
</tr>
<tr>
<td>7</td>
<td>ESH4 (Check)</td>
<td>4.5</td>
<td>7.5</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Injera consumer preference scale: 1 = poor; 9 = excellent
Drought tolerant hybrids

Summary of yield and injera making quality of candidate drought hybrids (2017-2021)

<table>
<thead>
<tr>
<th>No</th>
<th>Hybrids</th>
<th>Yield-all environment (2017-2021) (t/ha)</th>
<th>Injera consumer preference (1-9 scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>K19020</td>
<td>5.0</td>
<td>7.0</td>
</tr>
<tr>
<td>2</td>
<td>K19021</td>
<td>4.6</td>
<td>6.5</td>
</tr>
<tr>
<td>3</td>
<td>K19026</td>
<td>4.5</td>
<td>7.5</td>
</tr>
<tr>
<td>4</td>
<td>K7148</td>
<td>4.3</td>
<td>6.5</td>
</tr>
<tr>
<td>11</td>
<td>ESH5 (Check)</td>
<td>4.0</td>
<td>7.0</td>
</tr>
<tr>
<td>12</td>
<td>ESH4 (Check)</td>
<td>3.1</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Injera consumer preference scale: 1 = poor; 9 = excellent

Drought tolerant hybrids, Mieso 2021, Photo by Tamirat Bejiga
Wider-plot observation and seed multiplication test - 2022

- Wider plot observation conducted at two locations (Mieso and Kobo)
- Producibility of the hybrid (seed multiplication/synchronization test) at Melkassa
- Most of the candidate hybrids showed good field performance and synchronization
Flowering synchronization of candidate hybrids

<table>
<thead>
<tr>
<th>TRIAL</th>
<th>KEY</th>
<th>PEDIGREE</th>
<th>Flowering (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
</tr>
<tr>
<td>DUAL</td>
<td>K10550</td>
<td>Tx623A x PRL984104 MR732</td>
<td>67</td>
</tr>
<tr>
<td>DUAL</td>
<td>K10552</td>
<td>TX623A x PRL984098 SUCR36</td>
<td>70</td>
</tr>
<tr>
<td>DUAL</td>
<td>K10544</td>
<td>PU216A x PRL984104 MR732</td>
<td>80</td>
</tr>
<tr>
<td>DUAL</td>
<td>K10538</td>
<td>ATx631 x MR732</td>
<td>77</td>
</tr>
<tr>
<td>DROUGHT</td>
<td>K7148</td>
<td>PU207A X MR747</td>
<td>76</td>
</tr>
<tr>
<td>DROUGHT</td>
<td>K7411</td>
<td>P111535A X P9405</td>
<td>79</td>
</tr>
<tr>
<td>DROUGHT</td>
<td>K7410</td>
<td>P111535A X P9401</td>
<td>79</td>
</tr>
<tr>
<td>DROUGHT</td>
<td>K19020</td>
<td>P111199A x Sucr36 80/70</td>
<td>67</td>
</tr>
<tr>
<td>DROUGHT</td>
<td>K19026</td>
<td>P111283A x Sucr36 80/70</td>
<td>74</td>
</tr>
</tbody>
</table>

* Highlighted in yellow showed poor synchronization

➢ 2 dual (K10544 & K10538) and 2 drought (K7148 & K19020) hybrids selected for a final verification and release in 2023
Forage sorghum hybrid activities

Sorghum Sudan grass forage hybrids

- Evaluation of a set of 23 sorghum Sudan grass forage hybrids at three locations (Melkassa, Mieso and Kobo)

Sweet sorghum hybrids

- Evaluation of 27 Sweet sorghum hybrids and check cultivars at two locations (Mieso and Kobo)
5. Promote and support the development of a sorghum hybrid seed system in Ethiopia

Aims

- Farmers education on the agronomic and economic merit of hybrids
- Supportive programs for availability of quality hybrid sorghum seed production by both public and private agencies
- Market linkages to supply chains of quality hybrid sorghum seed and grain production and distribution
Demonstration of ESH4 and ESH5 hybrids

<table>
<thead>
<tr>
<th>Year</th>
<th>Region</th>
<th>Area planted (ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2019</td>
<td>Tigray, Amhara &amp; Oromia</td>
<td>124 demo plots</td>
</tr>
<tr>
<td>2020</td>
<td>Tigray</td>
<td>6.0</td>
</tr>
<tr>
<td>2020</td>
<td>Oromia</td>
<td>12.0</td>
</tr>
<tr>
<td>2021</td>
<td>Oromia</td>
<td>48.0</td>
</tr>
<tr>
<td>2022</td>
<td>Amhara</td>
<td>57.6</td>
</tr>
<tr>
<td>2022</td>
<td>Oromia</td>
<td>90.6</td>
</tr>
</tbody>
</table>
Large-scale demonstration plots of ESH4 and ESH5 hybrids

Mieso area, 2021
Demonstration of ESH5 to Farmers

ESH5 hybrid demonstration to farmers, Mieso 2021, Western Hararghe, Ethiopia
Demonstration of ESH4 to Farmers

ESH4 hybrid demonstration to farmers, Mieso October 2021, Western Hararghe, Ethiopia
Farmers Field day – Visit to demonstration plots

Large scale demonstration of ESH4 hybrid sorghum at Mieso October, 2022
Training to Private Seed Producer

Training to staff of Ethio-Agri CEFT engaged in large scale hybrid seed production (27 ha) in 2020
Human capacity development

Technical support to research staff through zoom meetings, free messaging and calling apps (Viber, WhatsApp)

• Techniques of setting up crossing blocks
• Foundation and certified hybrid seed production techniques
• Parental line development for hybrid breeding program and
• Development of mapping populations
Human capacity development

Technical support and guidance to graduate students

• Technical support and guidance to 6 MSc and 7 PhD students working on various topics in sorghum
Human capacity development

Training of seed experts at OSE

Training to seed experts from Oromia Seed Enterprise (OSE), Shashemene 2021, Ethiopia
Issues

- **Conflict in Northern Ethiopia**
  - The conflict in Northern Ethiopia (Tigrai, Amhara and Afar) caused significant disruption and loss of research capacity in the region.
  - Research stations affected:
    - **Tigray**: Humera, Shiraro stations and all on-farm activities – since 2020
    - **Amhara**: Kobo, Sirinka, Sekota, Jari, Chefa stations and all on-farms activities – 2021

- **Inflation**
Summary of key achievements

- QTLs associated with drought tolerance identified
- Large set of hybrids of Ethiopian landraces evaluated that has generated evidence on heterotic responses of the native germplasm
- Parental lines development reached an advanced stage for a hybrid program based on the native germplasm
- Candidate dual and drought hybrids close to the final stage of release
- Progress made in learning the discipline and practice of a hybrid seed production system at EIAR and some of the public and private seed enterprises
Planned activities for final year and way forward

- The sorghum program (EIAR) will take over the activities that need further work
  - Finish parental line development (landrace R and B-lines) – may need additional 1 and ½ year
  - Verification and application for release of candidate hybrids – 1 year (2023 main season)
- Some will continue as student activities:
  - Field-based drought data and thesis/paper write-up and QTL characterization (Chalachew)
  - Complete phenotyping for TE and RSA (Abel)
  - Complete Striga phenotyping and write-up (Abiy)
  - Complete heterotic data analysis and any follow-up field activity (Amare)
Project contribution to the sorghum value chain

- Good progress made in developing and promoting hybrid varieties that can strengthen the seed supply value chain and encourage the development of commercial seed enterprise systems in the country
  - Building a strong national sorghum research program
  - Farmers’ education
  - Engage and support seed enterprises
- Potential and brighter future for the seed enterprise systems to bring broader impact
Acknowledgment

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