Nitrogen Fixation and Disease working group
Summary of discussion and plans

1. Assessing strain effectiveness.

• Understand the contribution bacterial genotypes (species-level) to chickpea N-fixation. Need to decide on experimental design by summer 2016.

- ~10 major lineages of symbionts
- test at multiple locations in Ethiopia
- use prevailing host genotypes (small holder vs commercial operations might require different selections).
- Establish experimental design before end of 2015 and begin preparing thereafter. Implement plan during 2016 growing season. Coordinate with partners.

• N2 Africa will focus on understanding the interaction between legume and Mesorhizobial genotypes.

- which host genotypes?
- which bacterial genotypes?
- which phenotypes?
- How does one define and quantify strain effectiveness?
- which protocols?

Related activity: Aladdin Hamwieh is analyzing the effect of host genotype on nodule number.

• Sample within in India in collaboration with Indian partners.

- Complete sampling in 2015/16 growing season if possible.
- Plans were made to sample 5 agro-climatic zones, with up to 125 samples per zone (e.g. 5 sites x 25 samples per site to understand both local and regional variability).
- All samples will be collected as DNAs; extracted in India with USAID-purchased kits; sent to UCD for sequencing.
- A subset of samples will also be collected as bacterial strains isolated by Indian partners, principally at IARI in Dehli.
- Illumina sequencing and informatics to evaluate diversity will be conducted at UCD and USC.
- Goal is to have a subset of strains for field testing in the 2016 growing season -- ambitious, but doable.

- Need to identify local industry partner in India, equivalent to Manegesha Biotech LLC in Ethiopia.

2. Make new collections: Filling gaps in the collection.

- Sampling to be conducted this field seasson (2015)
- Extend current USAID project range by sampling north of Gonder, e.g., Tigray (Zehara Damtew?).
- Sample low pH soils in western Ethiopia in the context of Kassahun Tesfaye's PEER project (New PEER student?).
- Aladdin Hamwieh has collections of chickpea symbionts in Egypt that can be provided to the project.

3. Sequence and analyze the full set of strains currently under analysis by partners.

- Fassil Asefa at AAU has ~ 40 strains that have been phenotypically characterized.
- Asfaw Hailemariam at Menagesha Biotech LLC has strains that are in current use as inoculants.
- Endalkachew Woldemeskel and colleagues from N2 Africa have a set of chickpea strains, largely collected from southern regions.
- Strain collection at Holetta (need information from Ethiopian partners).
- Aladdin Hamwieh's collection from Egypt.

Strains will be sent to UC Davis as DNA (no permits required for import), not live cultures. The USAID project will provide the DNA extraction kits and either train the partners or perform the DNA extractions directly, as preferred.

4. Assays and criteria for assessing N-fixation

- Experimental considerations:
  - Aim for resilient and reliable outcomes from inoculation.
  - Establish efficacy using dominant chickpea varieties under field conditions.
  - Include reference strains in all assays to permit comparison among trials.
  - Should phosphate be added in assays?
  - Should strain occupancy be assessed relative to added inoculum?
- What data should be collected and how does one define "strain efficiency"

Current practices include:

-total N by means of Keldall
-comparison to controlled uninoculated
-Grain yield and quality
-Plant biomass
-Should we score phenology?
-Sample at grain filling when N demand is highest?
-Determine residual soil N?
-Understand cultivation history of each assay location
-Are nodule number and dry weight practical under field conditions? If so, then how should one interpret such data?

5. Student activities 2015-16

- Zehara's Damtew
    - Work in Fassil Assefa's lab to quantify in vitro phenotypes (e.g., phosphate solubilization, etc.)
    - Coordinate with Manegesha Biotech to develop standard inocula from strains to be used in field trials (see #1, above). UC Davis will cover costs, as feasible.
    - Development of field experiment design (see #1 above).

6. Work with partners to identify facility and infrastructure needs*.

- Lyophilizer
- -80 storage
- Other?

*Note that the USAID project does not have financial resources to meet infrastructure needs beyond those specified in the current budget. Nevertheless, having a credible list can provide targets when opportunities to obtain resources arise.

7. Diseases and Pests: Fusarium wilt, Ascochyta blight, seedling disease complex, pod borer.

- Ascochyta blight
    - Judith Lichtenzveig: Objective of country-wide collections and population genetics in Ethiopia. No collection established yet.
    - Genetic resistance in Kabuli, from ICARDA.

- Some resistance in Desi.
- No resistance has been systematically introgressed into preferred desi varieties of Ethiopia. Should this be done, or will landraces be abandoned non-systematically due to disease susceptibility?

• Fusarium wilt

- Negussie Tadesse and Aladdin Hamwieh (ICARDA).

They have made a collection of 120 isolates within Ethiopia. Koch's postulates are completed and some genotyping has been done. The USAID project can help with additional genotyping.

• Dagnachew Besha (EIAR)

- Dagnachew is a PhD student on the USAID project who will work on Fusarium. EIAR-AAU.

✓ Coordinate with Negussie and Aladdin to determine (1) what additional sampling might be conducted, if any, and (2) how the USAID project can help with genotyping.
✓ Work with UC Davis for F3 population development to facilitate Fusarium wilt screening in wild-derived material.

• Pod borer

Gashaw Sefara is a Masters student on the USAID project, EIAR associated with Hawassa University.

Explore possibility of an internship at ICRISAT with Hari Sharma. During the proposed internship, Gashaw coul screen wild parents or wild-derived F3 populations for pod borer resistance.

• Seedling diseases

Damping off is caused by a complex of soil fungi. No further discussion on this topic.

8. Communication