

Population Development working group: Summary of discussion and plans

A. Populations

A1. Types of populations

Three types of populations were discussed: **reverse introgression lines (revILs)**, **advanced backcross introgression lines (ABI)**, and **Early Generation Segregating populations (EGS; F3 families)**. revILs and ABI lines were part of the original research plan, while EGS lines were newly proposed during the meeting. The consensus of these discussions was that EGS populations are highly desirable because they will enable phenotyping in an accelerated time frame for a range of phenotypes of interest to the chickpea lab's internal activities and to affiliated partners. We will use the outcome of EGS phenotyping to prioritize specific wild x cultivated lineages for ABI development, rather than the broad, non-directed approach to ABI development previously envisioned.

A.1.i. *revIL* populations: activities, timelines.

Currently F1s are being advanced to F2s. Once physiologically mature, F2 seed will be genotyped by a dry seed chip extract and genotype protocol prior to planting for intercrossing. The primary trait and genomic region for revILs is the Dtf-QTL-Chr3 (the Days to Flowering QTL on Chr3), with a secondary emphasis on a plant architecture locus on Chr1.

An ***immediate activity for the Nuzhdin group at USC*** is to model crossing outcomes of F2 intercrosses, assuming that F2 parents are selected to be either (1) uniformly heterozygous for cultivated and wild alleles at the Dtf-QTL, or (2) one parent heterozygous and the other homozygous for the cultivated Dtf-QTL region. Questions to address are: (a) which zygosity is superior to increase recombination genome-wide and around the Dtf-QTL region, which will be reset to the cultivated state; (b) what is the fewest number of F2s needed to optimize recombination; and (c) do pairwise or diallel crosses provide the best route to maximizing recombination in the inter-crosses? Modeling can be benchmarked against the RAD-GBS data for the first F2 pilot populations, which is already analyzed and phenotyped.

A.1.ii. *EGS* populations: activities, timelines

F3 seed from the seed chip genotyped F2 plants will form the EGS populations. The purpose of the EGS populations is to permit assessment of genetic variation for particular traits within each wild-cultivated lineage. Those lineages demonstrating variation, particularly those with trait values that exceed those of cultivated parents or of other wild lineages, will become the focus of continued analysis and ultimately breeding. We will target 50 F3 for each wild-derived lineage. Given 20+ wild donors and 2 elites (for Ethiopia and India) this equates to ~1,000 plants/elite (~2,000 total) for field planting in the spring

2016 at the UC Davis research farm. EGS populations would be available for in-season field phenotyping in Ethiopia in August 2016 and in India in October 2016. Field (rather than greenhouse) planting of F2s will allow us to develop larger numbers of F3 families, and provide bigger F3 seed bulks to facilitate a wider range of phenotypic screens at partner sites.

B. Genotyping

B.1. Target QTL regions are Dtf-LG3 and GH-LG1 (Growth Habit). Pod shattering is explicitly not being addressed in the chickpea innovation lab's experimental populations, as we (1) do not know the genetic architecture (i.e. contributing genomic regions/QTLs) of this trait in chickpea to assimilate into the populations, and, (2) we need to avoid selecting too many loci to avoid complications of linkage drag. Shattering in the segregating populations can be dealt with using bridal veil bags to catch seed, if needed, or from periodic harvesting of pods as they mature and prior to acute dry down that fosters shattering.

B.2. Standardized protocols:

Genotyping: The decision was made to use the U of Saskatchewan's seed nicking protocol for genotyping during population development.

Marker platform: single locus analysis. Rather than gel-based genotyping of in-dels, SNPs will be used for normalizing to cultivated QTL regions. The SNPs for Dtf-LG3 and GH-LGH1 from UC Davis are already validated in U of Saskatchewan's populations and will serve as markers for phenology and growth habit.

Genome-wide genotyping via RAD-GBS: When (which population stage) to do RAD-GBS remains to be finalized? Based on training set data (F3s from NSF project's populations) and modeling it could be done with seed-chip gDNA of F2s with a modified protocol, or with leaf tissue derived gDNAs. It could also be deferred to the next round of inter-crosses (iF2)s.

C. Data management

The benefit of upfront tracking of pedigrees, derivatives, and samples was recognized. Within the chickpea innovation lab, GCP's Integrated Breeding Platform (IBP) was chosen as the platform for tracking pedigrees through phenotyping data (U of Saskatchewan has its own possibly proprietary system that would apparently be difficult to implement). IBP's BMS (Breeding Management System) advantages are that it is free-ware, is a long-term

platform developed for use by breeders and agronomists, and built to be compatible with Phenomics databases.

IBP offers short courses on its BMS typically in nearby countries. Two of the chickpea innovation lab trainees, Lijalem Korbu and Kassaye Negash, have attended a training workshop in Addis Ababa. Further training (or practice via implementation) is likely needed, and might require appropriate desktop or laptop computers that are currently specified in the EIAR sub-budget.