Project report (October 1 2022 to March 31 2023)

Project Title: Improving flood and drought tolerance for soybean in North Central region.

Participating institutions: University of Missouri, Kansas State University, University of Minnesota, Iowa State University

Objectives:

- 1. Characterize soybean germplasm for early season flood tolerance at seedling establishment and early vegetative growth stages and identify tolerant genetic resources and molecular markers for breeding applications.
- 2. Develop flood and drought tolerant germplasm and varieties in maturity groups 0 to IV for north central region by introgression of tolerance traits from the already developed genetic resource in late maturity groups (late IV and early V).

Research plan for Yr 1:

Obj. 1

• Screen for tolerance during seedling emergence and the early vegetative stages (separately) in a soybean core set (300 diverse lines with WGS data). The experiments will be conducted using soil-beds in the greenhouse of the University of Missouri (Dr. Nguyen) in Yr 1.

At the University of Missouri, 300 diverse lines with whole-genome resequencing data were tested for early season flood tolerance in our flooding channel in the greenhouse for 3 times. Each time, 5 biological replicates were planted for testing. Plants were subjected to flood stress for 8 days and flood injury scores were evaluated for each plant. Significant phenotypic variation in flood tolerance was observed. We selected 60 lines contrasting in flood tolerance level for further confirmation in the greenhouse in April 2023. We will further select 10 most tolerant and 10 most sensitive lines for field confirmation at 4 locations this summer.

Genome-wide-association-studies (GWAS) will be performed to identify loci associated with flooding tolerance and DNA makers for the tolerance genes/QTL will be designed and developed for marker-assisted selection. These results are expected to be updated in our next report.

Meanwhile, we validated our DNA markers for gene pyramiding. We are ready to genotype the breeders' crossing and backcrossing materials starting from April 2023.

Obj. 2

• Crossing and population development plan to incorporate flood and drought tolerance traits into MG 0-IV soybeans for the North Central region.

At the University of Missouri, four backcross populations were initiated for the flood tolerance genes and four backcross populations were initiated for the slow wilting (drought tolerance) genes in the 2022 winter nursery at Puerto Rico. We are expecting F1 seeds in May 2023 for genotyping. We will start backcrossing from these F1 plants. Forward breeding populations will be also advanced through these F1 plants.

In addition, we will make 3 backcrosses populations for FT and 3 backcrosses populations for SW. The donor parent for the FT will be S12-1362 (2 FT gene on chromosome 3 and 13). We will use NILs as the donor parent for SW (2 SW genes on chromosome 6 and 10). The recurrent parents will be elite lines in MG IV. BC1F1 plants carrying genes for FT and SW (using MAS) will be advanced and backcrossed to each recurrent parent if funding is continued in year 2. During the summer of 2023, we will make 2 to 3 crosses of elite lines (MG mid to Late IV) conferring drought tolerance (SW) with a FT elite line carrying the two FT alleles from S12-1362. Advancement of the populations will be advanced from the F1 to the F4 generation in the winter nursery if funding is continued in year 2.

At the Kansas State University, two backcross populations were initiated in the Fall 2022 greenhouse for the flood tolerance genes and one backcross population was initiated in the Fall 2022 greenhouse for the slow wilting (drought tolerance) genes. The F1 generation from these three crosses is growing in the Spring 2023 greenhouse. These plants will be genotyped in April, and the BC1F1 generation will be produced from plants carrying the appropriate alleles. In the Spring 2023 greenhouse, one additional backcross population will be created to incorporate the slow wilting genes. Four elite parents to use in the forward breeding effort have been selected and crosses will be made in the summer of 2023.

At the University of Minnesota, we successfully crossed the flooding and drought tolerant donor parents to two MN lines each. We have enumerated 18 successful cross pollinations. We expect ~36 putative F1 seeds. We will plant these into our summer crossing block, genotype them to confirm F1 status, and cross back to MN current parents to create the BC1F1 seeds. We will plant multiple dates of the recurrent parents to help ensure the F1s nick with the RPs. We see this as a significant success as it was not easy crossing these MG 4 and 5 donor lines with MG 0 and 1 MN parents.

At the Iowa State University, initial crosses for backcrossing breeding were made using Iowa State University elite material (MG2 and early MG3) and S12-1362 in winter nursery. The F1 seeds will be planted in Ames, IA in 2023, and crossed with the elite parents for the first backcross. Marker profiling for the flood and drought tolerance genes will be done in Missouri for backcrossing program.

For forward breeding, two crosses were made in winter nursery using an MG2 and early MG3 elite Iowa State University line and S12-1362 in fall 2022. F1 seeds were harvested and planted in winter nursery in March 2023 for generation advancement. F2 generation will be planted in Ames, IA in spring 2023, as a part of pure line development. Marker-assisted selection will be used through MU lab (Nguyen).

Other updates from forward crossing program – part of prior efforts:

Selections from advanced yield tests were made in fall 2022 based on yield and maturity data. Parental strain included flooding and flooding/drought tolerance. Evaluations for either drought or flooding have not yet been done but are planned for 2023 season.