First quarter (January 1, 2024, to April 31, 2024)

Project funded by North Central Soybean Research Program

Project title - Field phenotyping using machine learning tools integrated with genetic mapping to address heat and drought induced flower abortion in soybean.

Participating institutions – Texas Tech University, Kansas State University, University of Missouri, and University of Tennessee.

Goals & Objectives

Long-term Goal – Develop soybean cultivars with 20 to 30% lower flower abortion under favorable to challenging environmental conditions, leading to about 10-15% increase in yield potential.

Objectives (Year 2)

- Continue to explore the genetic diversity in flower abortion under different soil moisture and climatic conditions using a diverse set of landraces and elite genotypes.
- Improve the image-based field phenotyping system and deep-learning tools to document temporal dynamics in flower abortion and pod retention in diverse soybeans grown under field conditions.
- Identify molecular mechanisms controlling flower abortion under diverse climatic conditions.

Objective 1 - Explore the genetic diversity in flower abortion under different soil moisture and climatic conditions using a diverse set of landraces and elite genotypes

A diverse selection of 50 soybean genotypes, classified within groups III and IV, were meticulously chosen based on considerations of maturity and lodging scores from Year 1 multilocation studies. Seeds were provided by the University of Missouri for implementation of trails across multiple locations, namely Kansas State University (KS; Manhattan), Texas Tech University (TX; Lubbock), and The University of Tennessee (TN; Jackson), marking the beginning of the second experimental year of our project. All locations are currently in the preparatory phase for planting, scheduled in May.

This year, a significant advancement in our methodology involves the integration of QR codes (Figure 1) onto all plot labels across all locations. This technological enhancement streamlines imaging processing and storage procedures, facilitating efficient data management.

Each genotype will be planted in four row plots with a spacing of 30" between them. Within each row, seeds will be planted at a density of 8 seeds per foot. Furthermore, to ensure



Figure 1. Label containing a QRcode for tracing.

robustness and reliability of results, each experimental setup will be replicated three times within each location.

At Lubbock - TX we will incorporate two distinct irrigation regimes -100% ET and 50% ET leveraging the region's natural hot summers to induce heat stress. These irrigation treatments will be implemented via a sub-surface drip irrigation system. Drought stress (50% ET) will specifically start and persist throughout the flowering stage.

Meanwhile, in Jackson - TN, a greenhouse experiment will be conducted under stress conditions, focusing on four lines exhibiting low flower abortion rates and another four lines with high flower abortion percentages, based on year 1 findings. This targeted investigation aims to understand soybean response mechanisms that result in differential flower abortion under control and stress conditions.

Objective 2 – Improve the image-based field phenotyping system and deep-learning tools to document temporal dynamics in flower abortion and pod retention in diverse soybeans grown under field conditions.

At each location, uniform platforms for imaging are being constructed, ensuring consistency across all sites (Figure 2). The imaging process will start using a single GoPro Hero 11 camera, with additional cameras added as the crop matures, if necessary. Parameters for camera setup will remain consistent with those utilized in the previous year, ensuring continuity and comparability of data.

To streamline the data acquisition process, a Python program for generating QR codes and label IDs (Figure 1) has been developed for common use across all locations. Additionally, a program for detecting QR codes within the recorded videos has been successfully developed and will be integrated into the model in subsequent stages.

The forthcoming task involves the development of a program designed to extract videos from the cameras and organize them into location-specific folders, incorporating field specifications such as row numbers and the number of cameras per row. This program will greatly simplify the process of video collection and enhance data management



Figure 2. Platform for flower and pod imaging.

efficiency for the machine learning-based flower and pod tracking algorithms.

Furthermore, significant effort has been made in refining the accuracy of the pod and flower tracking models through rigorous training processes, aimed at maximizing precision and reliability in data analysis.

Objective 3 - Identify molecular mechanisms controlling flower abortion under diverse climatic conditions.

To identify the molecular mechanism involved in flower abortion, we have selected the most contrasting accessions from FY23 multi-location trials for bulk transcriptomics analysis under controlled greenhouse conditions. Eight accessions, four lines exhibiting low flower abortion rates and another four lines demonstrating high flower abortion rates, will be evaluated under controlled greenhouse conditions to eliminate the variation associated with environmental factors.

Following tissues will be carefully harvested from 3rd internode to ensure the developmental stage and flower position. Tissue samples including pedicel (stalk connecting to stem), flower bud,

partially opened, fully opened, and flower after anthesis (initiation of senescence) will be collected periodically. Following the sample collections, RNA will be extracted from at least 3 replicates/stage/sample and will be subjected to RNA sequencing using commercial vendor. Approximately, 144 samples (8 genotypes X 6 stages X 3 replications) will be collected. RNAseq data will be analyzed using the standard pipeline to identify differentially expressed genes as detailed in our publication Chen et al. (2016; PMID: 27486466).